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The role of pine oil in cattle fly sprays

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NOTE TO USERS

THE ROLE OF PINE OIL
IN
CATTLE FLY SPRAYS

BY

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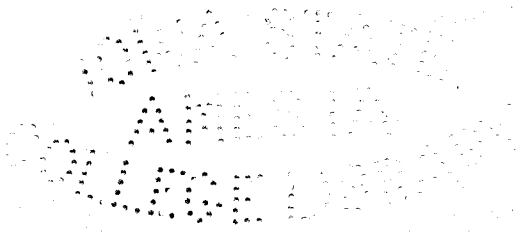

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TABLE OF CONTENTS

	Page
INTRODUCTION	4
REVIEW OF LITERATURE	5
DESCRIPTION OF MATERIALS	7
TOXICITY TESTS	10
Pine Oil	17
Pyrethrum	
Activation	18
Deterioration	41
Stabilization	42
Derris	
Activation	43
Rotenone	
Activation	52
Aliphatic Thiocyanate	
Activation	53
REPELLENCE TESTS	57
Pine Oil	62
Pyrethrum and Pine Oil	72
Pyrethrum	78
Derris	83

Derris and Pine Oil..... 84
Aliphatic Thiocyanate..... 90
Aliphatic Thiocyanate and Pine Oil..... 91
Fly Trend Observations..... 97
Physiological Effects..... 101
Discussion..... 103
SUMMARY AND CONCLUSIONS..... 105
Toxicity Tests..... 105
Repellence Tests..... 107
LITERATURE CITED..... 109
ACKNOWLEDGMENTS..... 112

INTRODUCTION

Spray formulae in general use against flies attacking cattle consist of a petroleum oil base, with which is incorporated some toxic and/or repellent ingredient such as, pyrethrum, derris, and pine oil. Certain synthetic materials have also been developed for this purpose.

This investigation was initiated and conducted with thought to the further utilization of pine oil in cattle fly sprays. The objective was to obtain quantitative data on the role of pine oil in petroleum oil base cattle fly sprays, both alone, and in combination with other commonly used ingredients (mentioned above). The results of this study, which was commenced February 19, 1934, at the Agricultural Experiment Station of the University of Delaware, Newark, Delaware, and was concluded September 30, 1935, are recorded herein. All the toxicity tests and, with one exception, all the repellence tests were conducted at Newark, Delaware. One series of repellence tests was conducted near New Castle, Delaware.

REVIEW OF LITERATURE

Pine oil has been used for a variety of industrial purposes, due mainly to its wetting ability. In the insecticide field, it has shown promise when employed for combatting a number of different insects.

Ginsburg (1927) found that pine oil increased the spreading power of mineral oils employed for the control of mosquitoes.

Parman, et al., (1927) reported that of the repellent materials tested against the screw-worm fly, certain pine products such as pine oil, crude turpentine, pine tar, and pine tar oil, were among the best.

Parman, et al., (1928) again found, when testing blowfly baits and repellents, that among the most efficient repellents were products obtained from the destructive distillation of the long-leaf pine such as, pine tar, pine oil, turpentine, and wood naphtha.

The work of Cory (1928a) indicated that pine oil materials have considerable promise in use against borers in fruit trees. Cory (1928b) also used pine oil emulsions, with and without pyrethrum, for the purpose of protecting cattle from flies.

Hartzell (1930) found that the use of pine oil showed promise of reducing materially the nicotine content of the

regular spray for pear psylla nymphs.

Laake, et al., (1931) made a study of the chemotropic responses of flies and concluded that pine tar oils were strongly repellent to all the species of flies dealt with, and some of these oils were effective in destroying flies when sprayed as a mist over them in barns, etc.

The work found to be most directly pertinent to this investigation was that of Freeborn and Regan (1932). In a study of fly sprays for dairy cows they concluded that pine oil increases the efficiency of petroleum oils, carrying pyrethrum, in proportion to the amount added.

Thompson and Worthley (1933) discovered a blend of various fractions of steam-distilled pine oils which was effective in field applications against codling moth larvae.

Information concerning pyrethrum and derris has become quite extensive. Gnadinger (1933) has treated the more important publications concerning the former, and Roark (1932) has given a digest of the literature of the latter.

DESCRIPTION OF MATERIALS

Base Oils. Three petroleum distillates, water-white in color and practically odorless and tasteless, served as base oils. They are referred to by numbers (30, 40 and 85) which designate their approximate viscosities. The specifications in each case, as furnished by the manufacturers, are given in Table 1.

TABLE 1. SPECIFICATIONS OF THE BASE OILS

Specifications	Base Oil Number		
	30	40	85
Specific Gravity	0.775/0.785	0.820/0.830	-
Saybolt Viscosity, 100° F.	30/35	40/45	85
Flash Point °F.	170/180	190	350
Fire Point °F.	190/200	-	400
Unsulphonatable Residue	98+%	95+%	95+%
Boiling Range °F.	370/490	410/700	-

Pine Oils. Pine oil and certain related materials, such as turpentine and rosin, are obtained from the distillation of pine stump wood. According to Pickett and Schantz (1934), "Pine Oil is the name given to a series of terpene alcohols, ethers, and ketones that are found in the heart wood and stumps

of pine trees." An approximate analysis of steam-distilled pine oil, as reported by them, is as follows:

Terpene hydrocarbons	5-10%
Borneol	5-10%
Fenchyl alcohol	5-10%
alpha Terpineol	50-60%
Other terpineols	15-25%
Terpene ethers	5-10%
Ketones and phenols	1-2%

Although various grades of pine oil differ in their alcohol and hydrocarbon content, commercial materials of dependable uniformity are nevertheless available. Two such products, known as "Yarmor" and "Middol", were given primary consideration in this study. The former is referred to as "No. 303" and the latter, as "No. 311". Several other pine oils, employed for comparative purposes, are mentioned in the text.

Pyrethrum Extract. A commercial pyrethrum extract, in No. 30 base oil, containing approximately 2.15 grams of pyrethrins per 100 c.c.,was incorporated in all formulae having pyrethrum as an ingredient. Five per cent by volume of this extract is considered to be equivalent to 1 pound of the flowers per gallon. Where the concentration is expressed in pounds, the solvent is understood to be in units of gallons.

Derris Extract. A commercial pine oil extract of derris, containing 5 per cent rotenone, was arbitrarily selected because of its convenience and commercial availability. In the literature there has appeared no explicit information as to the

proper solvent or concentration for a derris extract to be used in a cattle fly spray. The pine oil base in this extract was comparable in grade to No. 303.

Rotenone. A rotenone-pine oil concentrate was prepared by adding 10 grams of chemically pure rotenone crystals to 200 c.c. of No. 303. This material was allowed to stand for 36 hours at room temperature, with an occasional shaking. It was then decanted and kept bottled until used. A considerable quantity of the rotenone crystals remained undissolved. This solution was tested at a 2 per cent concentration.

Aliphatic Thiocyanate. A sample of a synthetic commercial insecticide, having as its essential toxic ingredient an aliphatic thiocyanate, was furnished by the manufacturer, who recommends it for use in fly sprays. This material is referred to simply as "aliphatic thiocyanate". It was arbitrarily selected as an example of an aliphatic thiocyanate insecticide, in order that some idea of the effect of pine oil upon such a material might be obtained.

TOXICITY TESTS

Experimental Insect. The house flies (Musca domestica L.) required for toxicity tests were bred in a small chamber maintained at temperatures ranging from 82° to 87° F. and at between 50 and 70 per cent relative humidity. The larvae were reared in Richardson's (1932) food mixture, consisting of wheat bran, alfalfa leaf meal, yeast, malt, and water. The emerged flies were caged (separately) each day, and were fed on a diet of milk, diluted 50 per cent with water. During the summer of 1934, fly breeding in the laboratory was suspended, and the new culture started in the fall of that year proved to be considerably less resistant to the insecticides under investigation. Five-day-old flies were used during the spring of 1934 and three-day-old flies, in all later tests. House flies were the only species employed in the toxicity tests.

Equipment. All toxicity tests were conducted in a spraying room (6' x 6' x 6'), which differed from that described by Peet and Grady (1928) in its larger windows and electric heaters (Plate 1). The heaters were of the strip type, and were located in opposite corners. Air was circulated from them by two 8-inch electric fans (Plate 2). A temperature of 85° F., plus or minus 1°, was maintained. A No. 25

Buffalo exhaust fan with a direct-drive, two-horsepower motor (Plate 3) was employed to remove the spray material from the chamber after each exposure period. The insecticides were sprayed from a De Vilbiss (The De Vilbiss Company, Toledo, Ohio) No. 5004 Special Atomizer. This atomizer had been adopted by the National Association of Insecticide and Disinfectant Manufacturers as official for such tests.

For collecting the "down" flies, a suction apparatus (Plate 4) was made from an electric hair dryer. Similar equipment has been used by a number of entomologists, for collecting other insects. To compare the results of "hand collection" versus "machine collection", two series of paired tests were conducted. Pine oil No. 303 served as the spray material. The recorded data are summarized in Table 2. Since no significant difference was found between these methods, the suction apparatus was used in all toxicity tests. With such a device the flies are collected much more rapidly and uniformly than by the older method.

TABLE 2. COMPARISON OF THE EFFECT OF TWO METHODS OF COLLECTION ON THE TOXICITY OF PINE OIL NO. 303 TO THREE-DAY-OLD HOUSE FLIES.

Method of Collection	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead			
			24 Hours	24-48 Hours	48-72 Hours	72 Hour Total
By Hand	10	100	77.3	13.4	1.2	91.9
Suction Apparatus	10	100	77.7	16.6	2.0	96.4

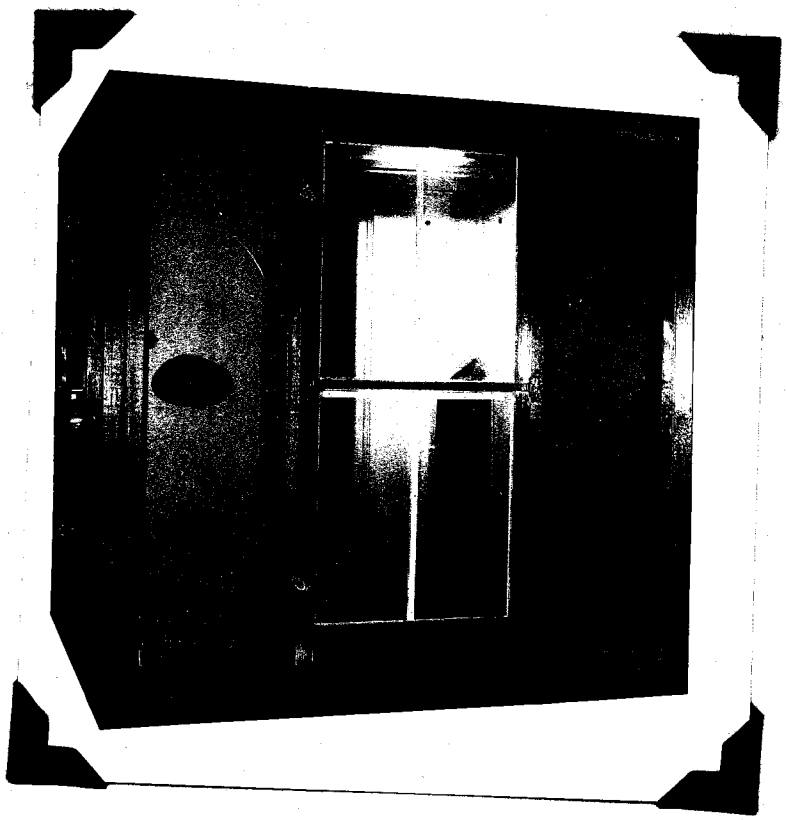

Plate 1. Spraying Chamber in which Toxicity Tests were Conducted.

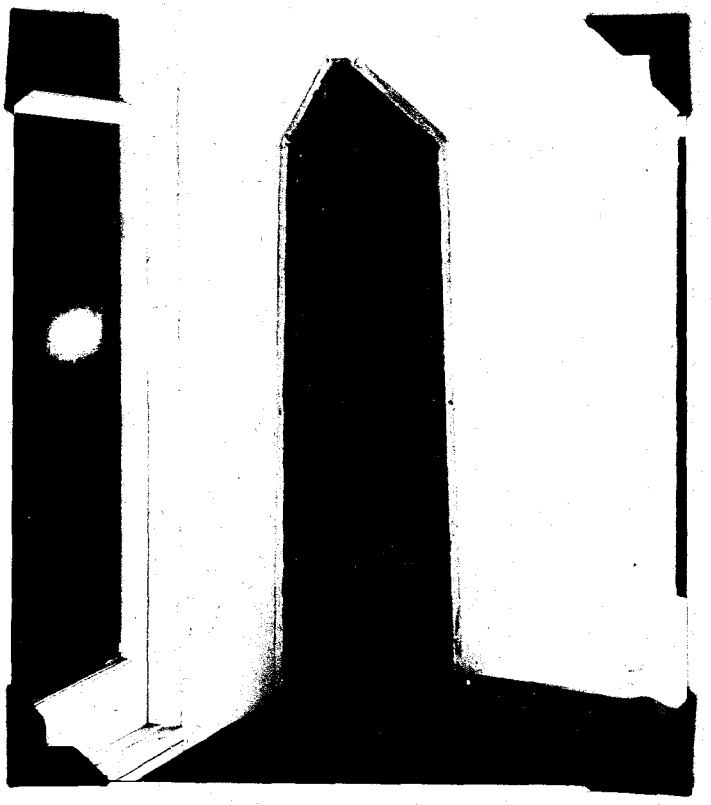

Plate 2. Interior of Spray Chamber Showing the Electric Heating Equipment.

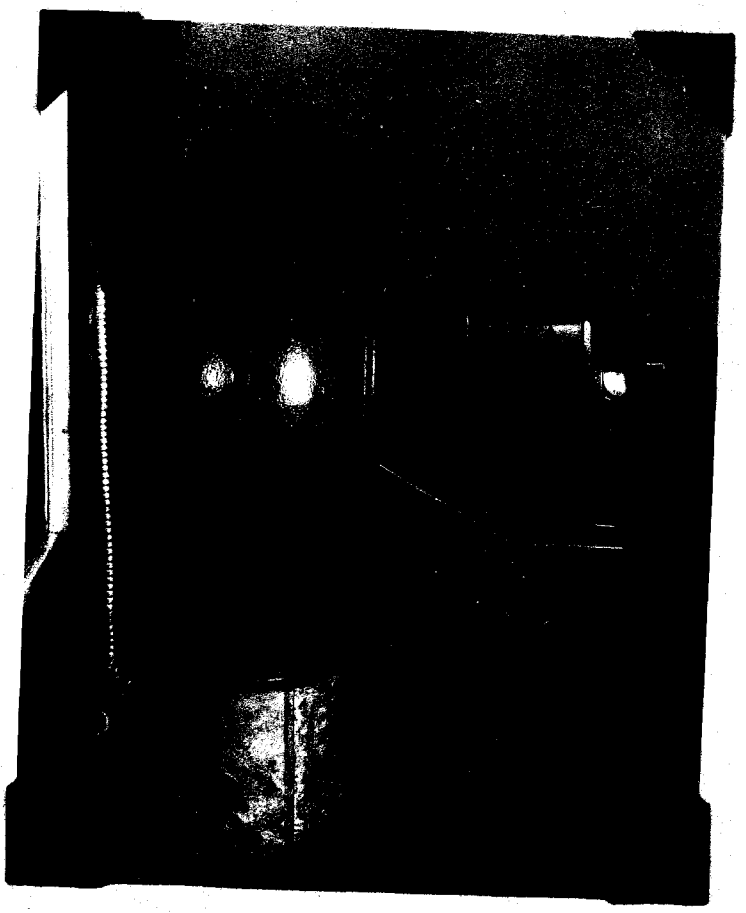

Plate 3. Fan Employed for Exhausting the Spray Chamber.

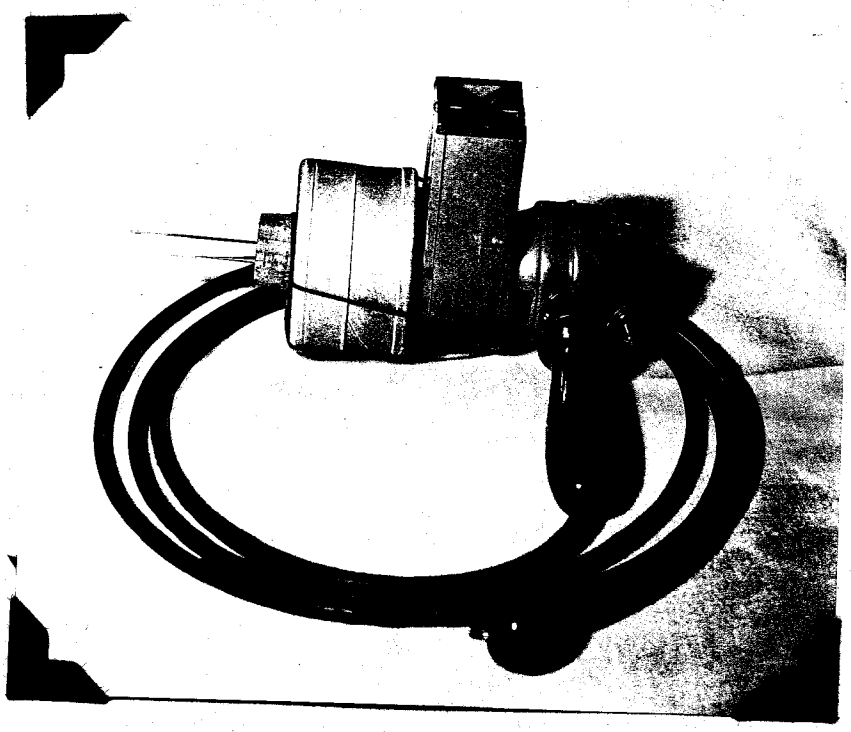

Plate 4. Electric Suction Apparatus Employed for Collecting the Disabled Flies.

Procedure. According to the "Peet-Grady" method (1928), about 100 flies are released inside the spraying room. Twelve c.c. of the insecticide are then atomized into it at 12.5 pounds air pressure. A ten-minute exposure period is allowed, after which the exhaust fan is started. The disabled flies are immediately collected and placed in an observation cage with food at their disposal. The number of flies on the floor is considered the "knockdown". After 24 hours, the number of flies dead is recorded as the mortality for the test.

This method was followed in its essentials. The only major departure was in the use of paper (Kraft No. 50) on the floor of the spraying room. The same paper was not used in more than two tests, and never for different formulae.

No satisfactory spray material has been established as yet for control tests with this method. Occasional tests with base oil alone showed no flies "down" after 10-minutes exposure. In order to place the spray formulae on an equal basis from the standpoint of fly resistance, the tests were conducted in pairs, using flies from the same cage for the corresponding tests in any given series of formulae. It has already been noted by H. H. Richardson (1933) that the resistance of flies varies from day to day.

TABLE 3. SUMMARY OF THE RELATIVE TOXICITY OF PINE OILS NO. 303 AND 311 IN BASE OIL NO. 85 TO FIVE-DAY-OLD FLIES.

Per Cent Pine Oil	Number of Tests	Pine Oil No. 303		Pine Oil No. 311	
		Per Cent Down in 10 Minutes	Per Cent Dead in 24 Hours	Per Cent Down in 10 Minutes	Per Cent Dead in 24 Hours
50	10	97.2	3.0	95.8	2.0
60	10	97.1	9.0	97.7	3.0
75	10	99.5	28.3	99.6	12.3
100	10	100.0	60.5	100.0	45.3

Pine Oil

Table 3 summarizes the results of eighty tests conducted to determine the toxicity of pine oil, using concentrations ranging from 50 to 100 per cent of Nos. 303 and 311 in No. 85 base oil. Figure 1 presents the toxicity curves from these data. It was found that pine oil stupefied a number of the flies that did not immediately fall to the floor. Consequently, all flies sufficiently affected to be readily captured were considered "down". While making the 24-hour mortality counts, it was observed that a considerable number of the insects were in a moribund condition. In order to obtain more complete mortality data, records were kept over a 72-hour period in all succeeding tests with pine oil.

It has been pointed out by Murphy and Peet (1934), that the toxicity of a given oil-base insecticide varies inversely with the viscosity of the oil. To ascertain the toxicity of pine oil when used with a base oil of lower viscosity (30-35 secs.), a series of tests was conducted with the same pine oils in No. 30 base oil. Tables 4 and 5 summarize this series. Figure 2 presents curves from the 24-hour mortality records and Figure 3 gives a comparison of the toxicity of Nos. 303 and 311 over a 72-hour period. The pine oils displayed a much greater toxicity when incorporated with the base oil (No. 30) of lower viscosity. In both cases No. 303 was more toxic than No. 311. The base oils No. 30 and No. 85 may be considered as defining the limits of a viscosity range within which the viscosity of any present day commercial cattle fly spray will fall.

Pyrethrum

Activation.

If a combination of pyrethrum (extract) and pine oil gives a higher per cent kill than the total effect of the two materials when used separately, it seems probable that one of the materials has produced an effect of "activation" upon the other, or upon the organism effected. For convenience, such an effect, or effects, is hereinafter referred to simply as "activation".

TABLE 4. SUMMARY OF THE TOXICITY OF PINE OIL NO. 303 IN BASE OIL NO. 30 TO FIVE-DAY-OLD FLIES.

Per Cent Pine Oil	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead 24 Hours	24-48 Hours	48-72 Hours	72 Hour Total
15	4	83.3	0.7	-	-	-
20	4	68.6	0.6	-	-	-
30	10	97.7	3.4	3.1	4.5	10.8
40	10	99.7	17.7	13.0	7.9	38.8
50	10	99.6	28.7	15.8*	8.9*	53.7*

*Data from eight tests only.

TABLE 5. SUMMARY OF THE TOXICITY OF PINE OIL NO. 311 IN BASE OIL NO. 30 TO FIVE-DAY-OLD FLIES.

Per Cent Pine Oil	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead 24 Hours	24-48 Hours	48-72 Hours	72 Hour Total
15	4	65.2	0.8	-	-	-
20	4	61.4	0.5	-	-	-
30	10	91.3	0.8	1.8	1.4	4.0
40	10	96.3	6.5	6.3	6.6	19.4
50	10	99.1	11.0	8.0*	8.3*	27.7*

*Data from eight tests only.

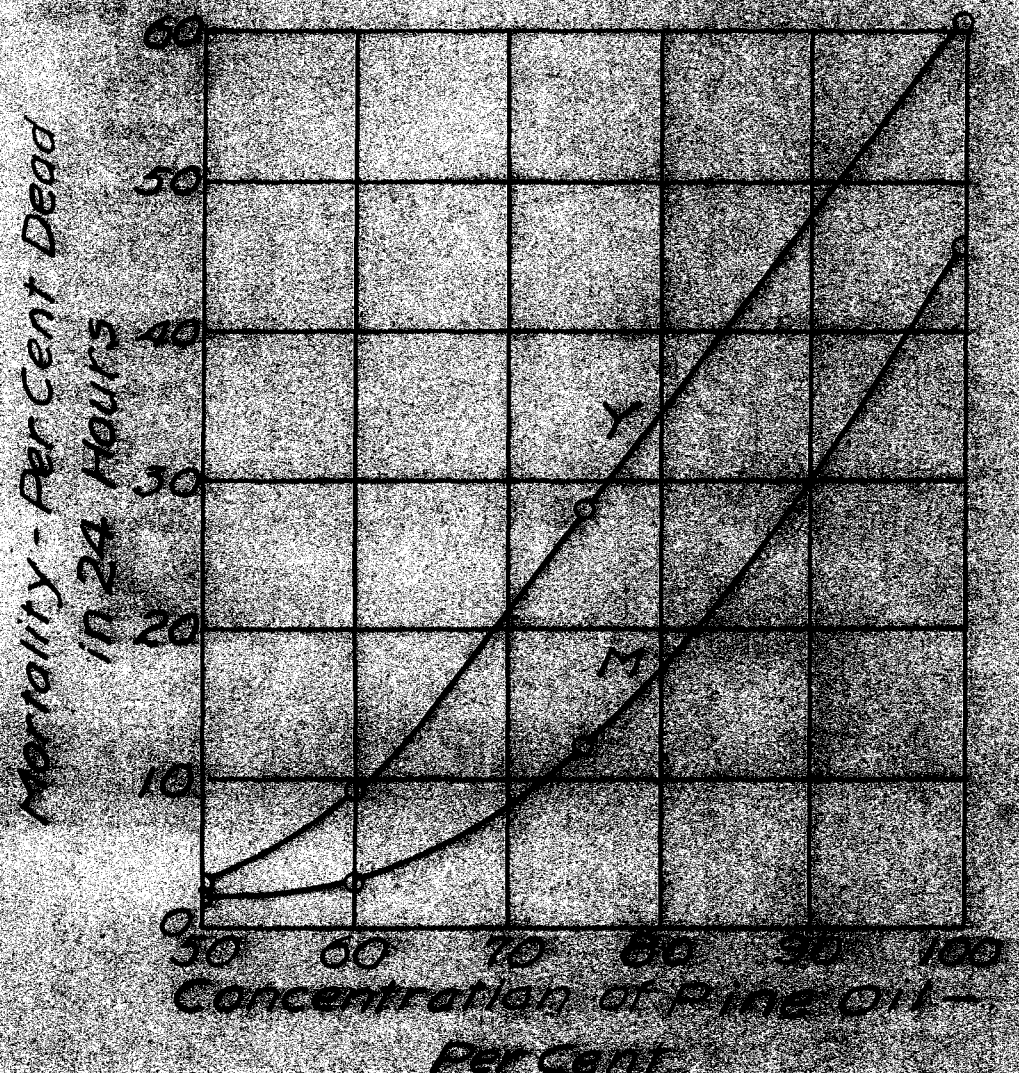

FIG. 1. TOXICITY OF PINE OILS NO. 303 AND NO. 311 IN NO. 85 BASE OIL TO FIVE-DAY-OLD BOLLS (Y=NO. 303; M=NO. 311).

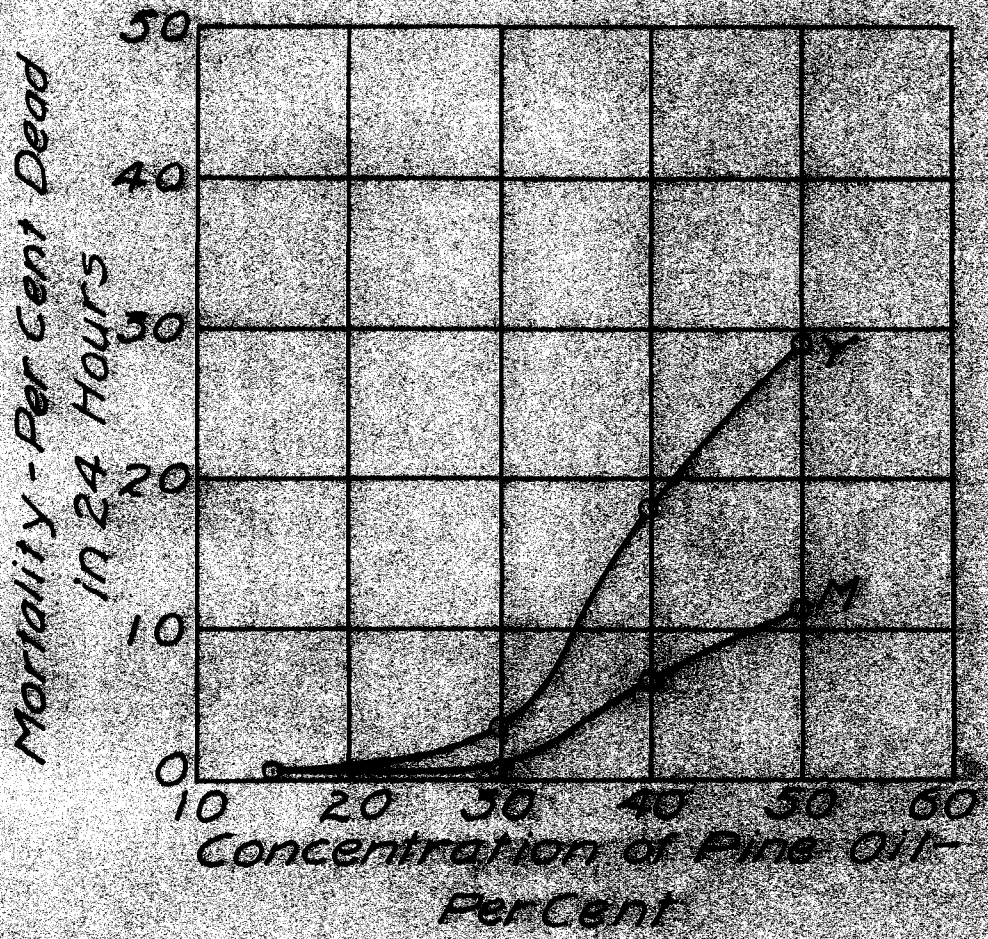

FIG. 2. TOXICITY OF PINE OILS NO. 303 AND NO. 311 IN BASE OIL NO. 30 TO FIVE-DAY-OLD FLIES (Y=NO. 303; M=NO. 311).

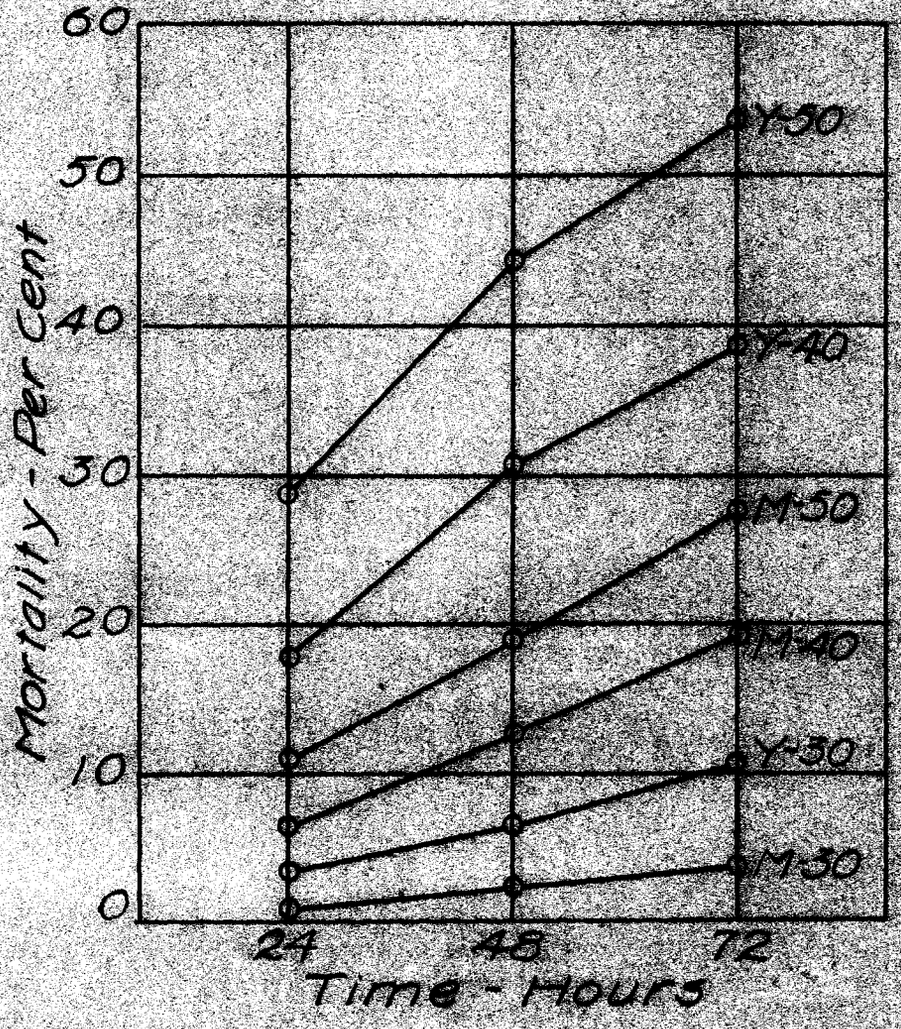

FIG. 3. TOXICITY OF PINE OILS NO. 303 AND NO. 311 IN NO. 30 BASE OIL TO FIVE-DAY-OLD FLIES (Y=NO. 303; M=NO. 311; NUMBERS REPRESENT PER CENT PINE OIL).

It is assumed that the material known to possess the greater toxicity, is activated by the other. It was apparent from the tests already conducted that pine oil was not sufficiently toxic to merit its use as the only added ingredient in an oil-base fly spray. Furthermore, pine oil No. 303 had proved distinctly more toxic than No. 311. For these reasons, it seemed desirable to determine the ability of No. 303 to activate pyrethrum. Four concentrations of pyrethrum were selected, 1/4, 1/2, 3/4 and 1 pound per gallon. A series of concentrations of No. 303 was added to each pyrethrum concentration.

In order to reduce possible variations caused by differences in the viscosity of the ingredients, No. 40 base oil was incorporated in these and all succeeding formulae. This oil has a viscosity (40-45 secs.) approximately identical with that of pine oils No. 303 and 311. The tests with the 1/4 and 1 pound concentrations of pyrethrum were conducted during the spring of 1934 and the remaining tests, in the fall of that year. Due to variation in fly resistance, the results with the different concentrations of pyrethrum cannot be compared directly, but tests in any one of the series should be, and are, in a logical relationship to each other. Tables 6, 7, 8, and 9 summarize, respectively, the results with the 1, 1/4, 3/4, and 1/2 pound concentrations (presented in the order conducted). Toxicity curves are presented in Figures 4, 5, 6, and 7. The activation effect produced by pine oil No. 303 is clearly shown with all four concentrations.

TABLE 6. SUMMARY OF THE TOXICITY OF 1 POUND PYRETHRUM PER GALLON WITH PINE OIL NO. 303 IN BASE OIL NO. 40 TO FIVE-DAY-OLD FLIES.

Per Cent Pine Oil	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead			
			24 Hours	24-48 Hours	48-72 Hours	72 Hour Total
0	10	75.0	12.3	2.2	1.9	16.4
5	10	94.4	18.3	2.9	2.0	23.2
15	10	99.9	29.0	3.3	3.1	35.4
25	10	99.9	36.7	5.2	3.4	45.4
50	10	100.0	82.9	5.8	3.8	92.5

TABLE 7. SUMMARY OF THE TOXICITY OF 1/4 POUND PYRETHRUM PER GALLON WITH PINE OIL NO. 303 IN BASE OIL NO. 40 TO FIVE-DAY-OLD FLIES.

Per Cent Pine Oil	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead			
			24 Hours	24-48 Hours	48-72 Hours	72 Hour Total
5	10	70.0	2.6	3.2	4.2	10.1
15	10	90.8	3.6	3.6	4.0	11.2
25	10	98.3	7.6	4.8	6.4	18.8
50	10	99.9	33.5	19.6	10.8	63.9

TABLE 8. SUMMARY OF THE TOXICITY OF 3/4 POUND PYRETHRUM PER GALLON WITH PINE OIL NO. 303 IN BASE OIL NO. 40 TO THREE-DAY-OLD FLIES.

Per Cent Pine Oil	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead			
			24 Hours	24-48 Hours	48-72 Hours	72 Hour Total
0	10	88.3	20.3	1.9	1.2	23.4
5	10	98.5	26.0	2.6	1.3	30.0
15	10	100.0	40.4	2.3	1.7	44.5
25	10	100.0	52.2	2.0	1.1	55.3
50	10	100.0	84.2	3.1	1.9	89.3

TABLE 9. SUMMARY OF THE TOXICITY OF 1/2 POUND PYRETHRUM PER GALLON WITH PINE OIL NO. 303 IN BASE OIL NO. 40 TO THREE-DAY-OLD FLIES.

Per Cent Pine Oil	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead			
			24 Hours	24-48 Hours	48-72 Hours	72 Hour Total
0	10	63.4	13.7	1.3	0.7	15.8
5	10	85.3	18.5	1.3	1.4	21.4
15	10	99.7	29.1	1.3	0.8	31.3
25	10	100.0	37.0	1.3	1.6	39.9
50	10	100.0	78.0	2.6	2.3	82.9

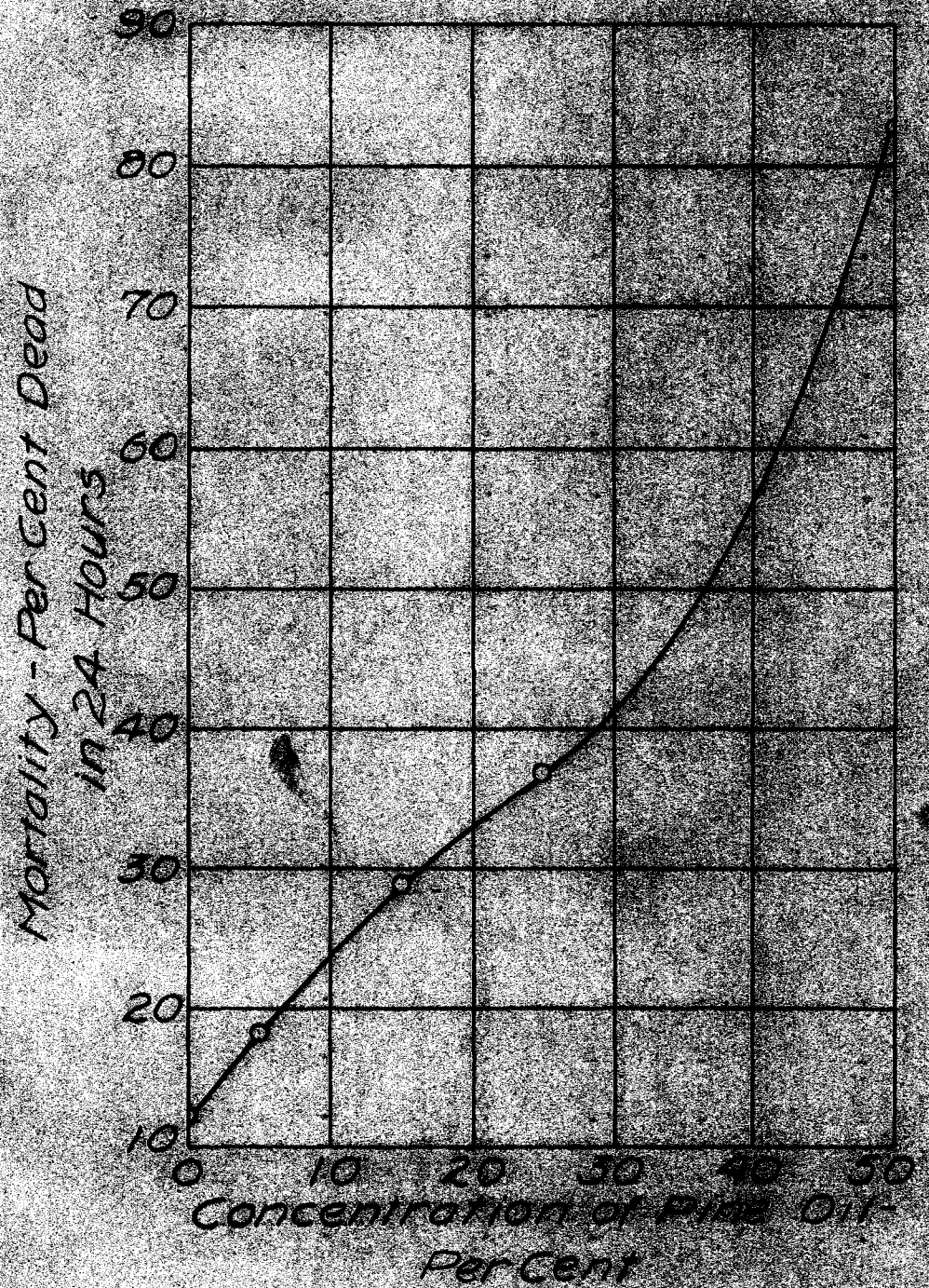

FIG. 4. ACTIVATION OF 1 POUND PYRETHRUM PER GALLON WITH PINE OIL NO. 505 IN BASE OIL NO. 40

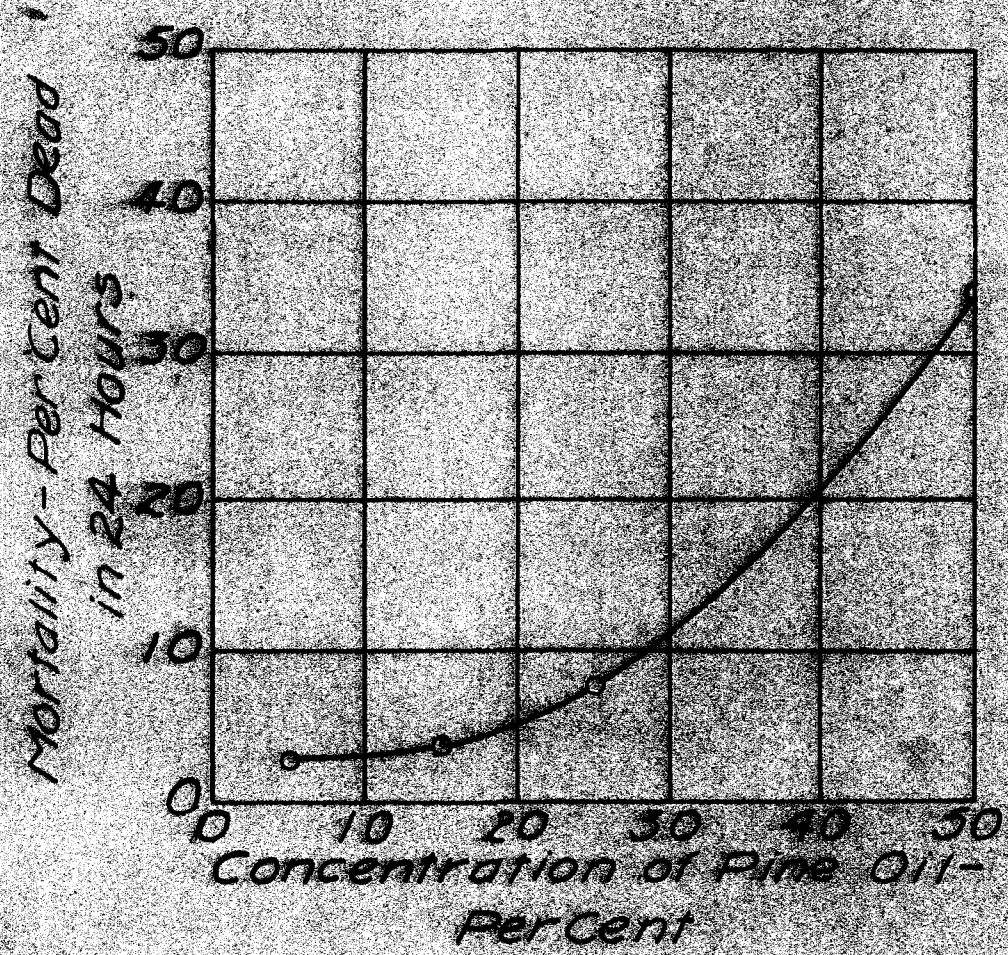

FIG. 5. ACTIVATION OF 1/4 POUND PYRETHRUM PER GALLON WITH PINE OIL NO. 303 IN BASE OIL NO. 40.

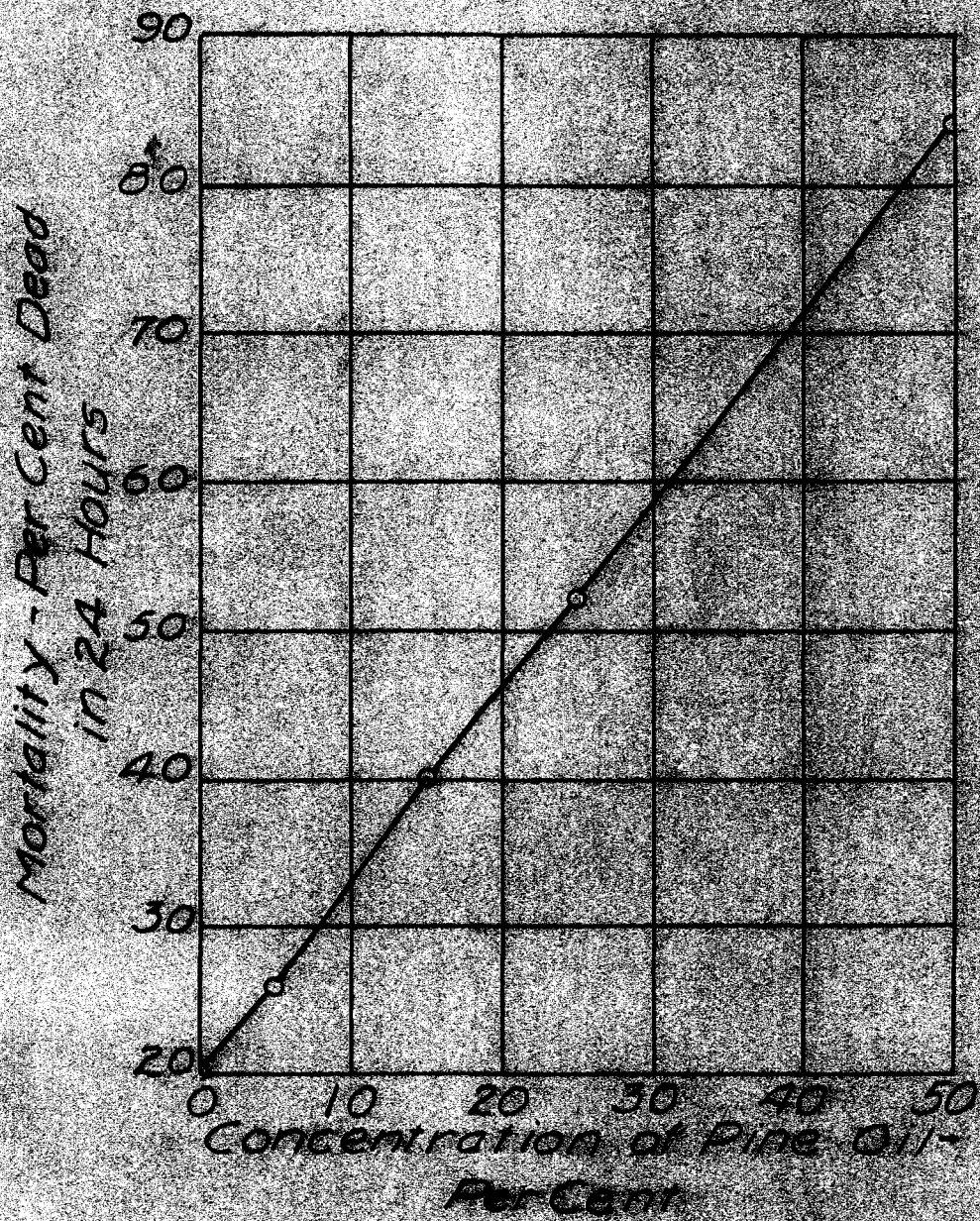

FIG. 6. ACTIVATION OF 3/4 POUND PYRETHRUM PER GALLON WITH PINE OIL NO. 303 IN BASE OIL NO. 40.

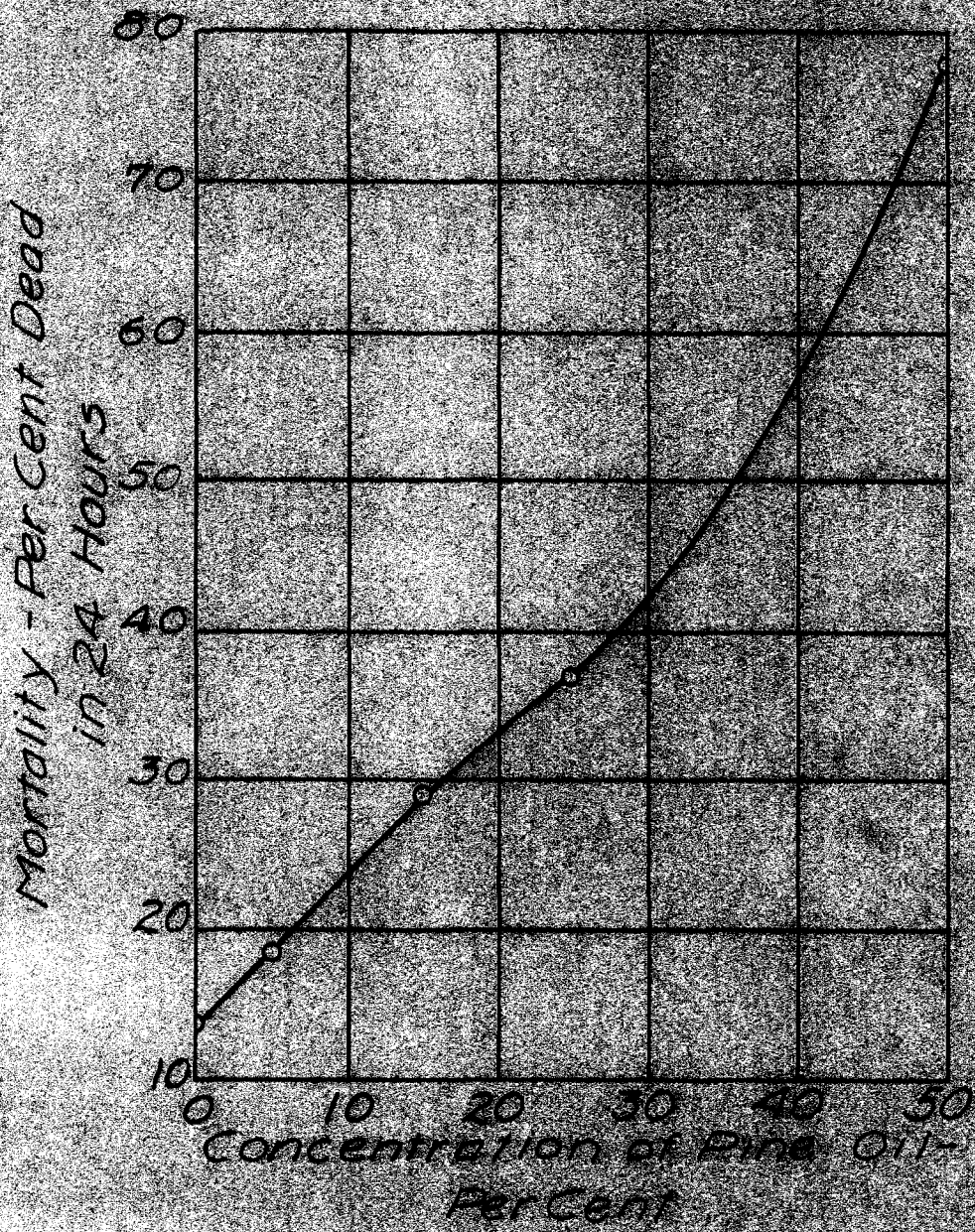

FIG. 7. ACTIVATION OF 1/2 POUND PYRETHRUM PER GALLON WITH PINE OIL NO. 503 IN BASE OIL NO. 40.

A series of paired tests was then conducted in order to obtain relative values for the four concentrations of pyrethrum alone. A summary of this series is given in Table 10, and the mortality curve for these data is presented in Figure 8, Curve B. Tests were also conducted with a series of "unknown" samples, furnished by the Committee on Insecticide Standardization of the National Association of Insecticide and Disinfectant Manufacturers, for the purpose of correlating the results obtained by various laboratories. At a later date, the constituents of these samples were learned. The results obtained with the three concentrations of pyrethrum in this series are summarized in Table 11. Figure 8, Curve A, presents the mortality curve for these data. A kerosene served as the base oil in the "unknown" samples.

TABLE 10. SUMMARY OF THE TOXICITY OF PYRETHRUM IN BASE OIL NO. 40 TO THREE-DAY-OLD FLIES.

Amount of Pyrethrum Per Gallon	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead in 24 Hours
1/4 lb.	10	52.0	19.9
1/2 lb.	10	67.4	15.9
3/4 lb.	10	80.7	24.1
1 lb.	10	86.7	37.7

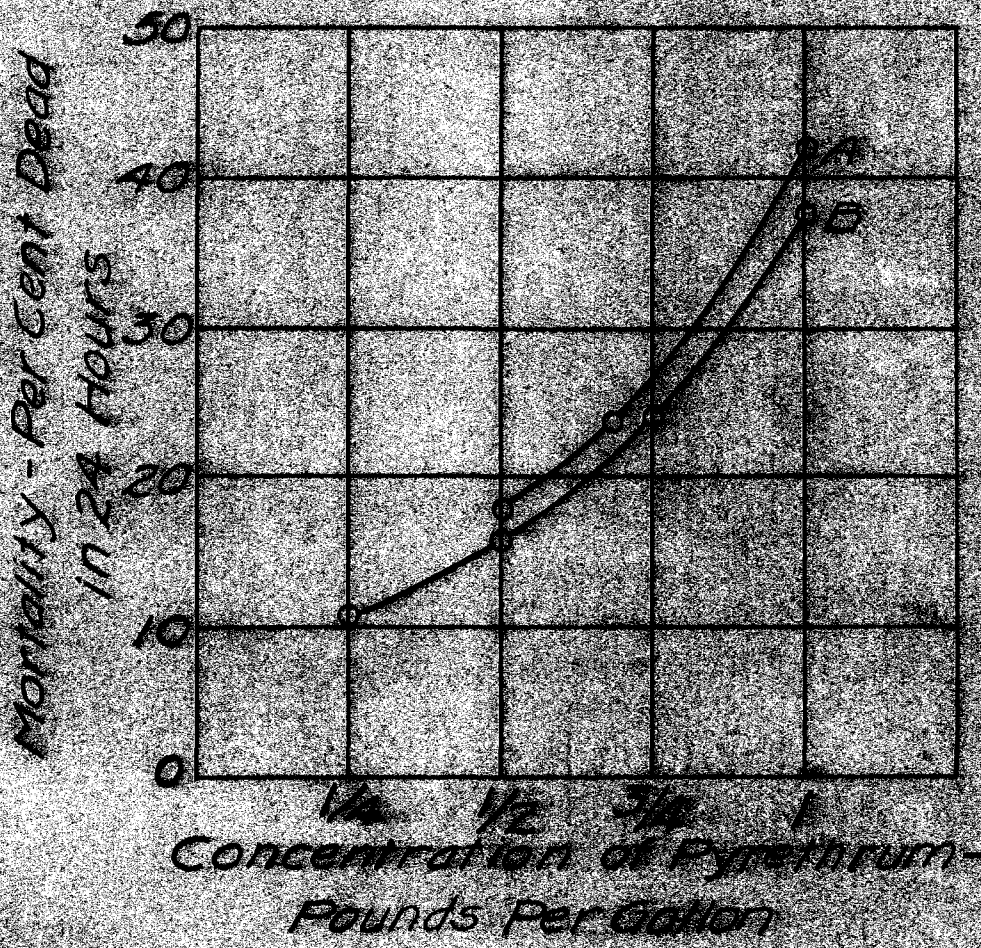

FIG. 8. TOXICITY OF PYRETHRUM EXTRACT TO THREE-DAY-OLD FLIES (A--IN KEROSENE BASE OIL; B--IN BASE OIL NO. 40).

TABLE 11. SUMMARY OF THE TOXICITY OF PYRETHRUM IN A KEROSENE BASE OIL TO THREE-DAY-OLD FLIES (N.A.I.D.M. CORRELATION SERIES).

Amount of Pyrethrum Per Gallon	Number of Tests	Per Cent	
		Down in 10 Minutes	Dead in 24 Hours
0.50 lb.	7	99.7	17.7
0.67 lb.	7	100.0	23.7
1.00 lb.	7	100.0	42.2

In both of these series with pyrethrum alone, a consistent relationship was found between concentration and mortality.

In the pyrethrum activation tests it was assumed that since pine oil No. 303 had proved more toxic than No. 311, it was also probably superior as an activator. In order to determine the relationship between high and low grade pine oils and their ability to activate pyrethrum, four formulae were tested. Each consisted of 3/4 pound pyrethrum per gallon, to which was added 10 per cent of the specified pine oil. Four pine oils, Nos. D100, K100, 303, and 309, ranging from low to high grade materials (in the order presented) were incorporated in these formulae. The results are given in Table 12. The relative"knock-down" and 24-hour mortality records are

presented in Figure 9. In this figure, the value designating the "probable toxicity of pyrethrum alone" has been determined by computation, i.e., in previous tests, with 3/4 pound pyrethrum and No. 303, it was found that pyrethrum alone produced approximately 60.5 per cent as great a kill (24 hours) as the same quantity of pyrethrum plus 10 per cent No. 303. On this basis, a value of 41.8 per cent was obtained for 3/4 pound pyrethrum alone. If the four pine oils be rated as to their grade, the same rating indicates their relative positions as activators of pyrethrum. However, no significant improvement was obtained with the material (No. 309) of higher grade than No. 303.

Apparently certain constituents of the higher grade pine oils, which were absent, or present to a lesser degree, in the lower grades, were playing an important part in the activation process. To investigate further this condition, a series was conducted in which two of the more important

TABLE 12. SUMMARY OF THE TOXICITY OF 3/4 POUND PYRETHRUM PER GALLON WITH FOUR DIFFERENT PINE OILS (10%) IN BASE OIL NO. 40 TO THREE-DAY-OLD FLIES.

Pine Oil Number	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead After 24 Hours	Probable Toxicity of Pyrethrum Alone	Per Cent Activation Produced
D100	10	84.9	51.6	41.8%	9.8
K100	10	93.1	62.6	41.8%	20.8
303	10	96.1	69.1	41.8%	27.3
309	10	95.5	69.8	41.8%	28.0

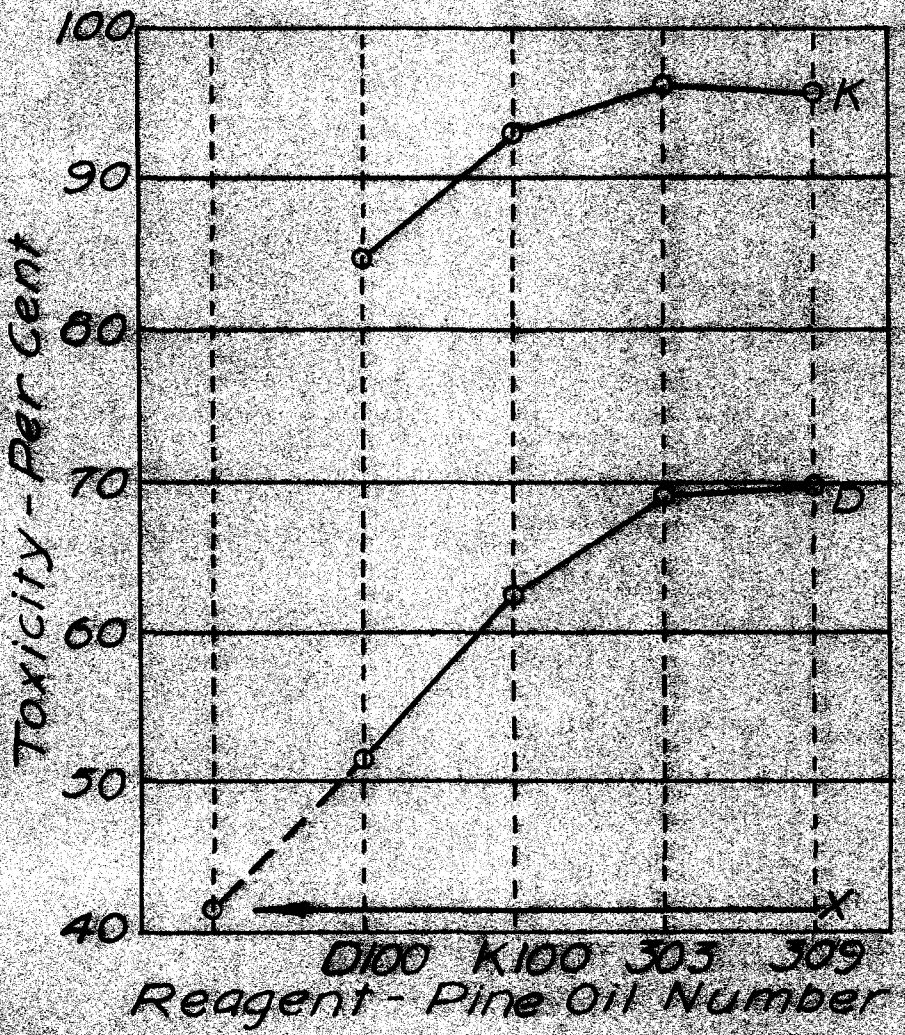

FIG. 9. ACTIVATION OF 3/4 POUND PYRETHRUM PER GALLON WITH FOUR DIFFERENT PINE OILS (10%) IN BASE OIL NO. 40 (K=DOWN IN 10 MINUTES; D=DEAD IN 24 HOURS; X=PROBABLE TOXICITY OF PYRETHRUM ALONE).

constituent fractions of pine oil, designated as Reagents A and B, were compared with pine oil No. 303 as an activator of 3/4 pound of pyrethrum. Each activator was added at a 10 per cent concentration, and a "pyrethrum alone" formula was included as a control. A summary of the results is given in Table 13. Figure 10 presents the relative "knockdown" and 24-hour mortality data. Reagent B produced an effect similar to pine oil No. 303, but the performance of Reagent A was less favorable.

From the activation tests it was evident that pine oil No. 303 might be substituted, in part, for pyrethrum in a spray material without loss in efficiency. Data were desired, therefore, on the relative toxicity of some practical formulae demonstrating this principle. The following combinations were selected for comparison:

Formula No.	1.	1 lb.	pyrethrum	per	gal.					
" "	2.	3/4 "	"	"	"	plus	10%	No.	303	
" "	3.	1/2 "	"	"	"	"	15%	"	"	
" "	4.	1/4 "	"	"	"	"	25%	"	"	

Table 14 summarizes the data obtained with these materials. Figure 11 presents their relative toxicity over a 72-hour period. All three of the pyrethrum-pine oil combinations proved superior to the "pyrethrum alone" spray, both as to "knockdown" and final mortality. This substantiates, with practical formulae, the fundamental principle of the activation of pyrethrum with pine oil, discovered in previous tests. From an economic viewpoint, each of the three combination sprays are less expensive

TABLE 13. SUMMARY OF THE TOXICITY OF 3/4 POUND PYRETHRUM PER GALLON WITH SEVERAL REAGENTS (10%) IN BASE OIL NO. 40 TO THREE-DAY-OLD FLIES.

Reagent	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead 24 Hours	Per Cent Dead 24-48 Hours	Per Cent Dead 48 Hour Total	Per Cent Activation Produced
Check	10	51.3	22.6	0.2	22.9	0
A	10	71.0	33.8	1.0	34.9	12.0
303	10	85.6	46.0	1.3	47.4	24.5
B	10	89.7	44.9	2.5	47.5	24.6

TABLE 14. SUMMARY OF THE RELATIVE TOXICITY OF FOUR PRACTICAL CATTLE FLY SPRAY FORMULAE TO THREE-DAY-OLD FLIES.

Formula Number	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead 24 Hours	Per Cent Dead 24-48 Hours	Per Cent Dead 48-72 Hours	Per Cent Dead 72 Hour Total
1	8	90.8	35.9	0.5	0.9	37.4
2	8	98.9	39.5	1.0	0.7	41.3
3	8	99.0	41.1	1.2	1.3	43.8
4	8	99.0	37.0	2.6	1.8	41.4

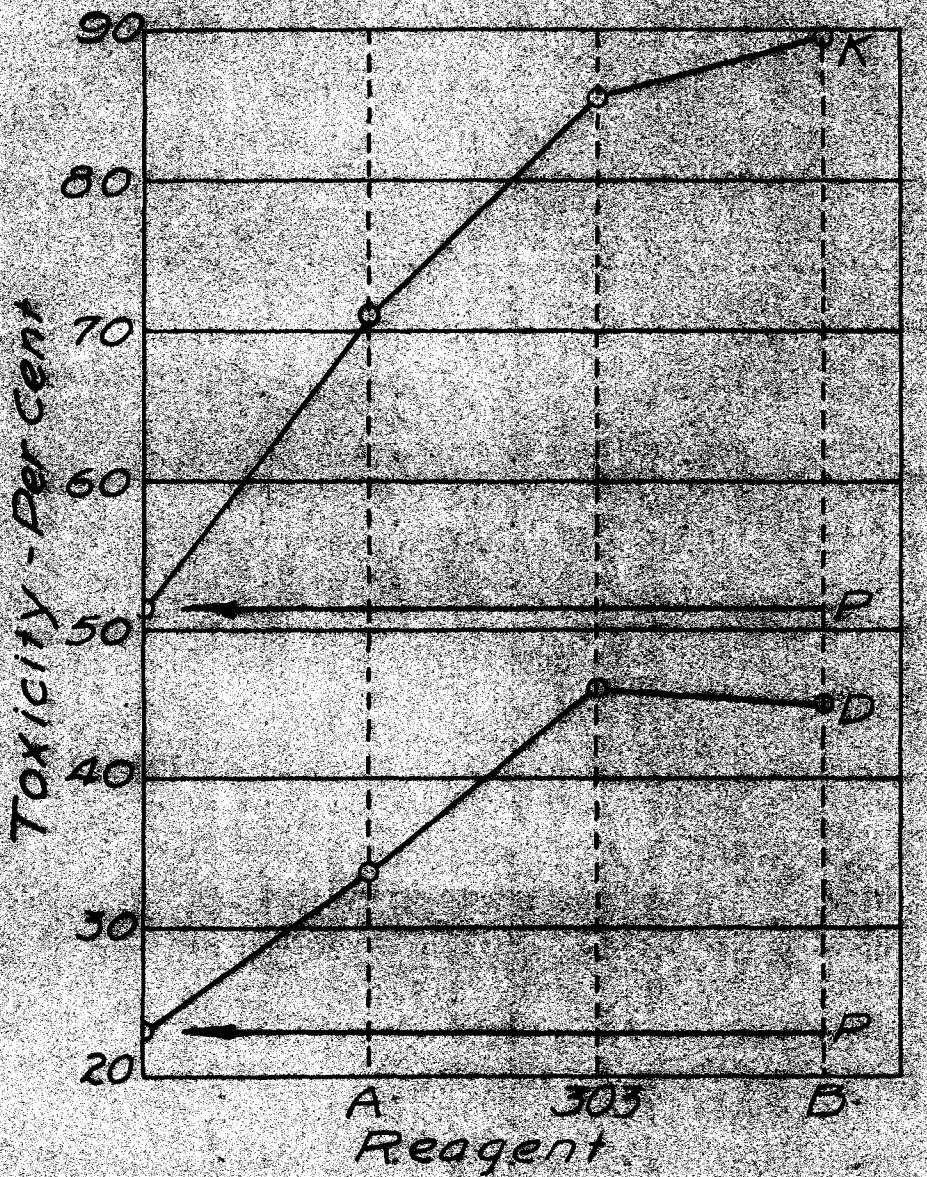

FIG. 10. ACTIVATION OF 3/4 POUND PYRETHRUM PER GALLON WITH SEVERAL REAGENTS (10%) IN BASE OIL NO. 40 (K=DOWN IN 10 MINUTES; D=DEAD IN 24 HOURS; P=PYRETHRUM ALONE).

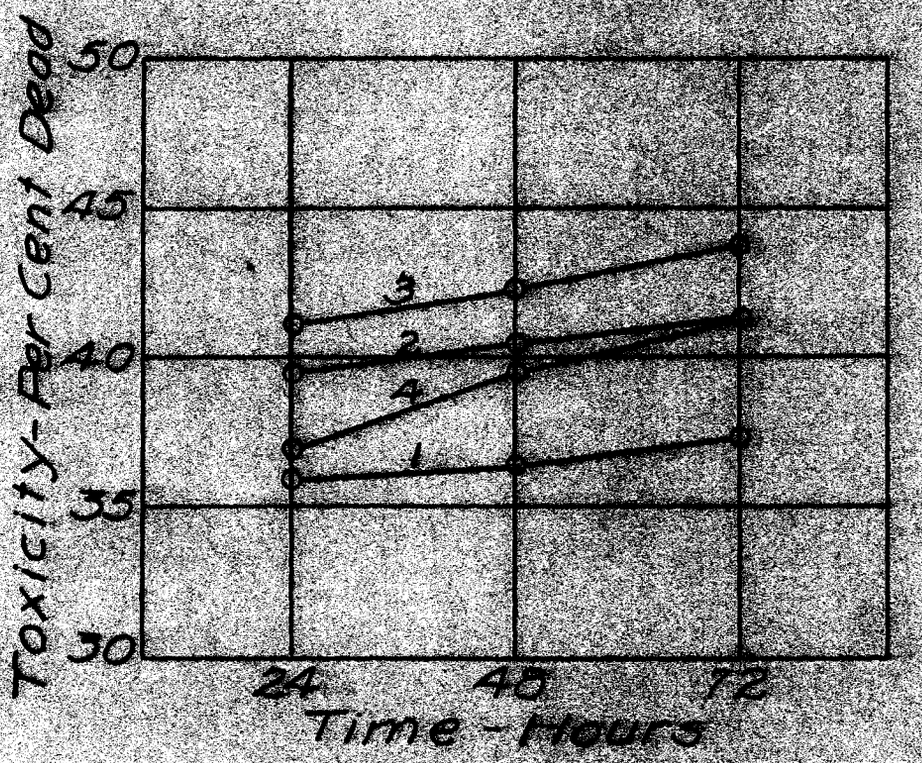

FIG. 11. TOXICITY OF FOUR PRACTICAL CATTLE FLY SPRAY FORMULAE OVER A THREE-DAY PERIOD; FORMULAE INDICATED BY NUMBER.

to manufacture than is the proprietary "pyrethrum alone" formula.

In order to compare these results with those obtained elsewhere samples of the four formulae were submitted to five commercial laboratories for "Peet-Grady" testing purposes. The average of the mortality results (24 hours) from all six laboratories was: Formula No. 1, 65.4 per cent; No. 2, 65.3 per cent; No. 3, 67.0 per cent; and No. 4, 64.8 per cent. It is apparent, therefore, that pine oil No. 303 can be substituted (in part) for pyrethrum in a cattle fly spray, without loss in toxicity, and with a simultaneous reduction in the total cost of the ingredients.

It is interesting to note that the pyrethrum-pine oil combinations show the quick-acting, toxic effect of pyrethrum alone, i.e., all significant mortality occurs within 24 hours. In addition, they always produce a higher per cent "knockdown". Consequently, "pyrethrum alone" sprays show a closer relationship between original "knockdown" and final mortality. The flies killed by pyrethrum (alone) apparently are those which never recover from the "knockdown", while with 100 per cent pine oil No. 303, all of the flies are definitely "knocked-down", but within 4 to 6 hours nearly all of them have partially recovered, show activity, and a majority are able to find the milk-soaked cotton in their cages. Mortality then slowly manifests itself, extending beyond 24 hours, with many

flies dying upon the cotton.

It was thought that the activation of pyrethrum produced by pine oil might be partially attributed to a reduction of surface tension, caused by the addition of pine oil. The results of surface tension determinations, given in Table 15, definitely eliminate this explanation. The activation effect may be due to either the wetting ability of the pine oil or to an inherent toxic property of the pyrethrum-pine oil combination.

TABLE 15. SURFACE TENSION DETERMINATIONS* (RING METHOD, CENCO-duNOUY TENSIOMETER)

Spray Material**	95% No. 40: 5% No.303	90% No. 40:10% No.303	80% No. 40:20% No.303	70% No. 40:30% No.303	45% No. 40:50% No.303
Temperature Degrees C.	29.5	29.5	29.5	29.5	29.5
Surface Tension Observed	30.2	30.4	30.4	30.8	31.9
	30.3	30.4	30.4	30.8	31.8
	30.2	30.3	30.5	30.8	31.7
	30.2	30.4	30.4	30.7	31.8
Mean	30.2	30.4	30.4	30.7	31.8

*These determinations were obtained through the courtesy of the Hercules Powder Company Experiment Station.

**Each solution contained 5 per cent of a pyrethrum extract, equivalent to 1 pound of flowers per gallon.

Deterioration.

Pyrethrum oil-base fly sprays lose toxicity upon prolonged exposure to sunlight. Three formulae were prepared in order to study the effect of pine oil upon the rate of deterioration. Each of them contained 3/4 pound of pyrethrum per gallon; the first served as a control, and to the second and third were added, respectively, 5 per cent and 15 per cent pine oil No. 303. Approximately three pints of each material were prepared, placed in flint glass jars, and exposed to sunlight in a greenhouse. Samples for toxicity tests were withdrawn at 15-day intervals. Ten "Peet-Grady" tests (paired) were conducted with each sample for each time interval. Table 16 summarizes the relative deterioration results, on a percentage basis. The "pyrethrum alone" spray showed a loss of 50 per cent in toxicity after 30 days exposure, but none in the following 30

TABLE 16. SUMMARY OF THE DETERIORATION OF 3/4 POUND PYRETHRUM PER GALLON IN BASE OIL NO. 40 WHEN EXPOSED TO SUNLIGHT IN A GREENHOUSE.

Per Cent Pine Oil	No Exposure	Loss in Relative Toxicity* After Exposure			
		15 Days	30 Days	45 Days	60 Days
0	0	14%	50%	50%	50%
5	0	0	0	0	15%
15	0	0	0	0	5%

*Calculated from per cent dead after 72 hours.

days. The two formulae containing pine oil No. 303 displayed no loss in toxicity until after 60 days exposure. On a percentage basis, the loss then was inversely proportional to the amount of pine oil present.

Gnadinger and Corl (1932) gave data on the deterioration of pyrethrum-kerosene base sprays (1 pound per gallon) in various containers. In flint glass bottles, exposed to sunlight, they reported a 50 per cent reduction in toxicity after thirteen months exposure. From their results it might be assumed that no further deterioration of the "pyrethrum alone" formula would take place within the first year of exposure.

Stabilization.

While conducting the deterioration of pyrethrum tests it was observed that, in the early stages of deterioration, the loss in toxicity of the solutions was apparently correlated with the rate of precipitation and loss of color, especially the former. Loss of the characteristic color of pyrethrum appeared to precede precipitation, which continued for a much greater period of time. The formulae containing pine oil No. 303, after complete loss of color, but no precipitation, showed no loss in toxicity. Consequently, it appeared that observations on the rate of color loss and precipitation would be a possible satisfactory means of rapidly testing a large number of materials as potential stabilizers of cattle fly sprays con-

taining pyrethrum. With this object in view, a number of formulae were prepared, placed in flint glass bottles, exposed to sunlight in a greenhouse, and observed periodically. The materials tested consisted of a number of pine oils and related products.

The tests indicated that the pine oils and alcohols were favorable sources to draw from, but the hydrocarbons were decidedly unfavorable. One of the lowest grade pine oils, contrary to those of higher grade, produced, instead of prevented, precipitation after three weeks of exposure in the laboratory (not sunlight). All of these materials were tested at a 5 per cent concentration with pyrethrum at the rate of 1 pound per gallon. No pine oil was found to be superior to No. 303.

The method employed in the stabilization of pyrethrum tests is inaccurate for distinguishing the merits of closely related materials, and is applicable only to the early stages of deterioration. After 28 days of exposure most of the formulae had reached such a condition that it was impractical to rate one above another.

Derris

Activation.

Activation tests with derris extract were conducted accord-

ing to the same procedure followed in the pyrethrum tests. Formulae containing 1 per cent and 2 per cent extract (Rotenone content = 1-2000 and 1-1000, respectively) were each tested with a series of concentrations of pine oil No. 303. Table 17 summarizes the results with the 1 per cent concentration and Figure 12 presents curves for the "knock-down", 24- and 48-hour mortality data. The records for the 2 per cent concentration are likewise given in Table 18 and Figure 13. From these results it is obvious that derris extract is activated by pine oil No. 303 at an even greater rate than pyrethrum. Furthermore, the low "knockdown" of derris extract, which is one of its greatest handicaps for use in fly sprays, is increased by the addition of No. 303 more rapidly than is the 24-hour mortality. If toxicity alone be considered, the combination of derris extract with pine oil No. 303 appears more promising than the pyrethrum-pine oil combination.

In the activation tests with pyrethrum it was found that four pine oils of different grade varied in the amount of activation produced. These same four pine oils (10 per cent) were also incorporated in formulae with 1-1/2 per cent derris extract. Table 19 summarizes these results and Figure 14 presents the "knockdown", 24- and 48-hour mortality data. In this figure, the point indicating the probable toxicity of the extract alone was determined by computation from the results of previous tests.

TABLE 17. SUMMARY OF THE TOXICITY OF DERRIS (1% DERRIS-PINE OIL EXTRACT) WITH PINE OIL NO. 303 IN BASE OIL NO. 40 TO THREE-DAY-OLD FLIES.

Per Cent Pine Oil	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead			
			24 Hours	24-48 Hours	48-72 Hours	72 Hour Total
1	10	35.4	20.4	10.8	0.4	31.7
5	10	48.6	26.3	16.6	1.4	44.4
15	10	67.2	38.5	22.6	1.2	62.4
25	10	79.5	46.4	27.5	1.0	74.9

TABLE 18. SUMMARY OF THE TOXICITY OF DERRIS (2% DERRIS-PINE OIL EXTRACT) WITH PINE OIL NO. 303 IN BASE OIL NO. 40 TO THREE-DAY-OLD FLIES.

Per Cent Pine Oil	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead			
			24 Hours	24-48 Hours	48-72 Hours	72 Hour Total
2	10	63.4	46.8	15.3	0.3	62.5
5	10	74.4	56.8	15.3	0.5	72.7
15	10	90.6	70.3	17.7	0.4	88.4
25	10	96.2	72.4	21.8	0.1	94.4

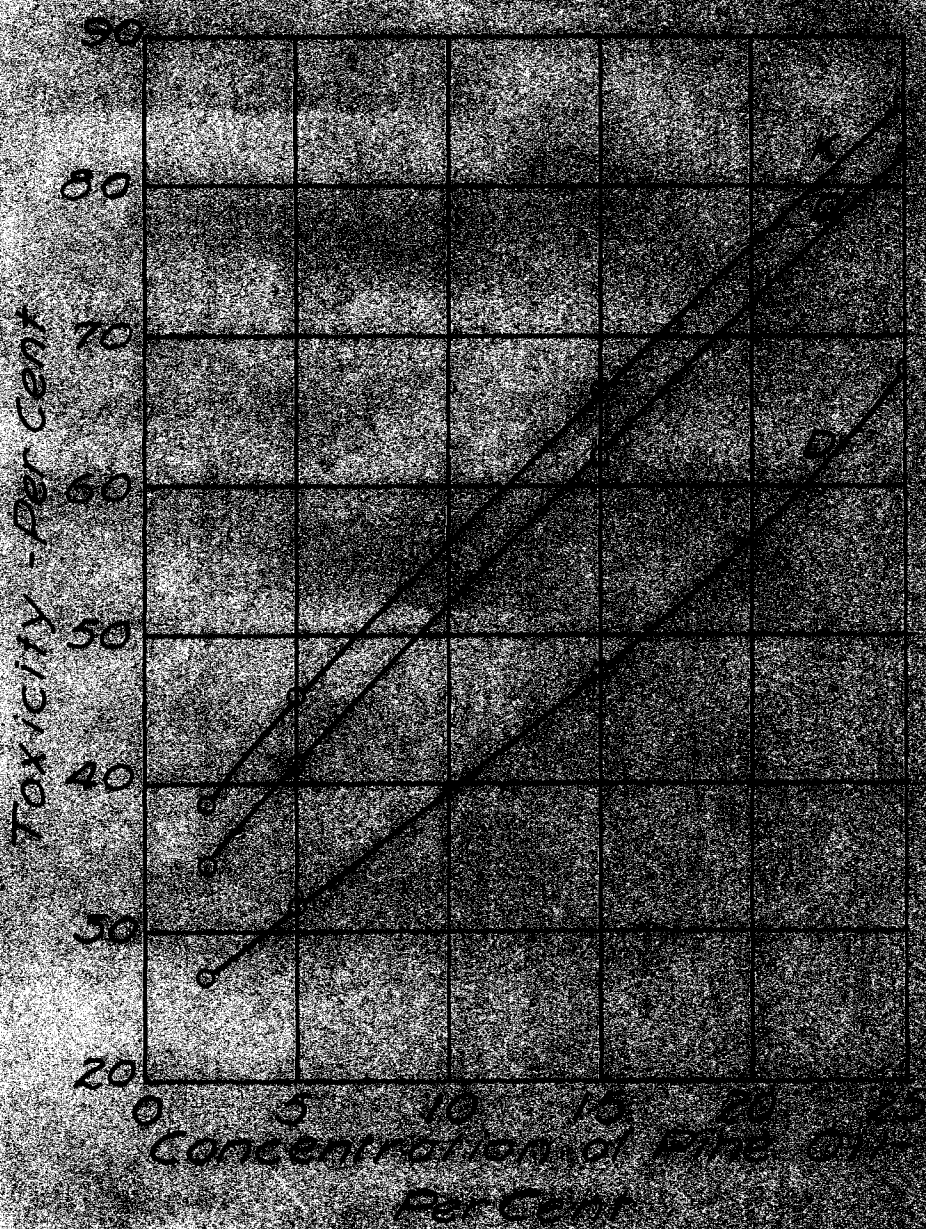

FIG. 12. ACTIVATION OF DERRIS (1% DERRIS-PINE OIL EXTRACT) WITH PINE OIL NO. 5 AND PINE OIL NO. 46 (RE-DONE IN 10 MINUTES, DEAD IN 24 HOURS; DERRIS IN 48 HOURS)

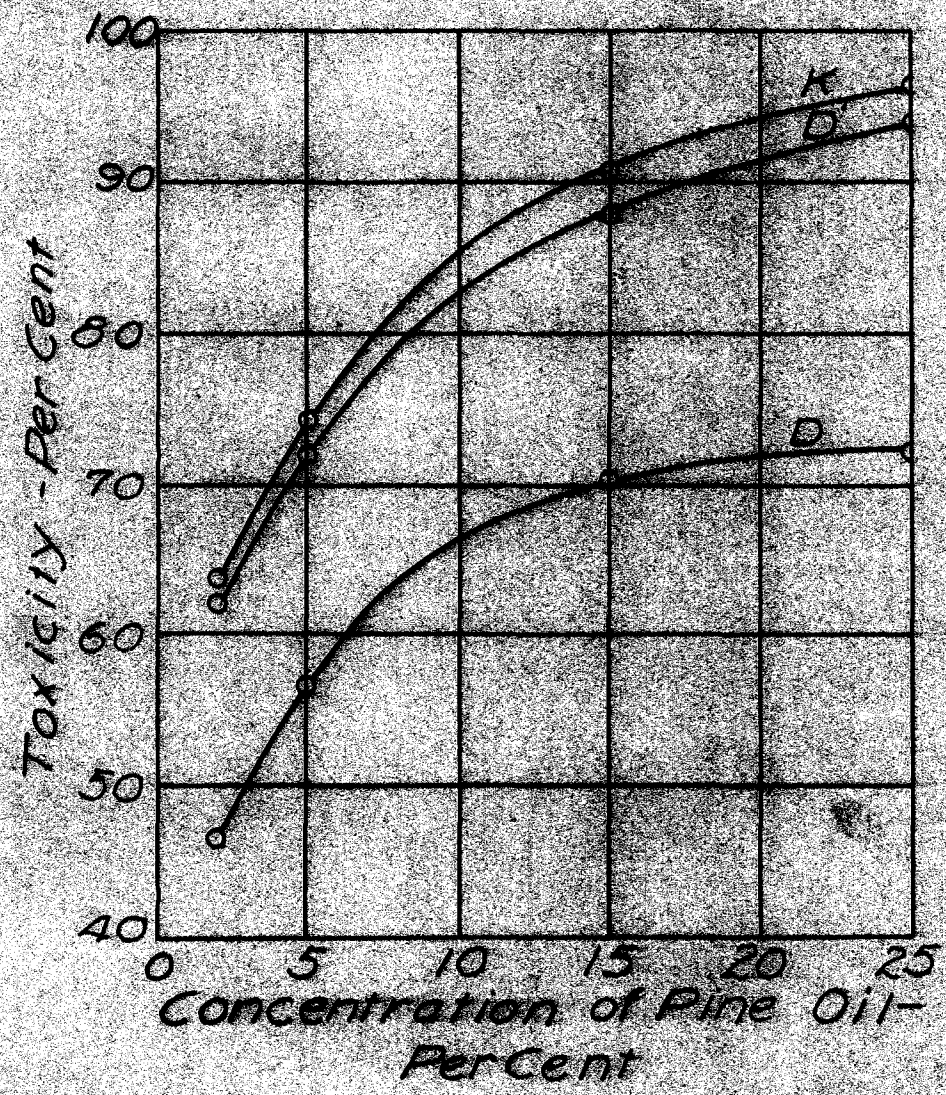

FIG. 13. ACTIVATION OF DERRIS (8% DERRIS-PINE OIL EXTRACT) WITH PINE OIL NO. 303 IN BASE OIL NO. 40 (K=DOWN IN 10 MINUTES; D=DEAD IN 24 HOURS; D^1=DEAD IN 48 HOURS.

TABLE 19. SUMMARY OF THE TOXICITY OF DERRIS (1-1/2% DERRIS-PINE OIL EXTRACT) WITH FOUR DIFFERENT PINE OILS (10%) IN BASE OIL NO. 40 TO THREE-DAY-OLD FLIES.

Pine Oil Number	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead 24 Hours	24-48 Hours	48-72 Hours	72 Hour Total
D100	10	60.6	45.5	13.5	0.3	59.4
K100	10	68.2	49.4	17.4	0.5	67.3
309	10	71.6	52.3	17.1	0.7	70.3
303	10	73.7	56.4	15.7	0.6	72.7

Further tests with 1-1/2 per cent derris extract were conducted to determine the relative activation merits of Reagents A and B (10 per cent). The results are summarized in Table 20, and Figure 15 presents the relative "knockdown", 24- and 48-hour mortality data.

From these tests it appears that the four pine oils and Reagents A and B, when used at a 10 per cent concentration, do not differ greatly in their ability to activate derris extract (1.5 per cent). A much greater difference was found in these materials when used with pyrethrum (3/4 pound per gallon). Apparently pyrethrum is a more sensitive indicator than derris for these several activating agents. None of the materials were found to be superior to pine oil No. 303, in respect to both cost and efficiency.

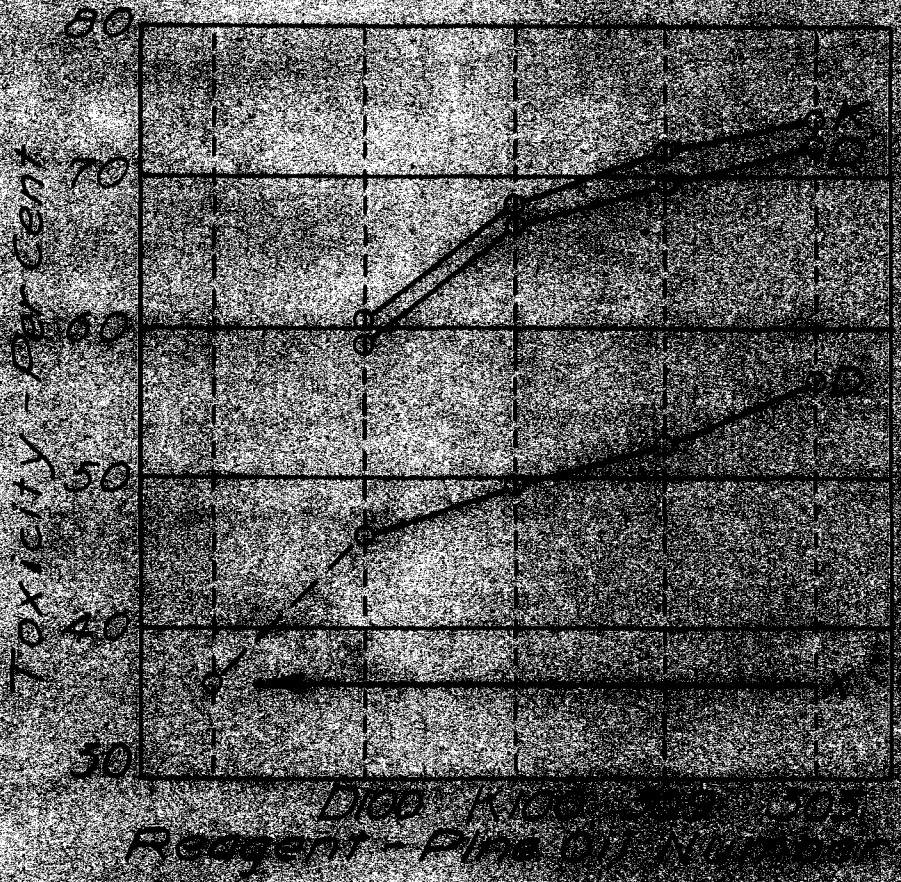

FIG. 14. ACTIVATION OF DERRIS (1-4 PER CENT EXTRACT) WITH [illegible] EXTRACT) WITH [illegible] IN BASE OIL NO. 40 [illegible] DEAD IN 24 HOURS. [illegible] PROBABLE TOXICITY [illegible]

TABLE 20. SUMMARY OF THE TOXICITY OF DERRIS (1-1/2% DERRIS-PINE OIL EXTRACT) WITH SEVERAL REAGENTS IN BASE OIL NO. 40 TO THREE-DAY-OLD FLIES.

Reagent	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead 24 Hours	24-48 Hours	48-72 Hours	72 Hour Total
A	10	70.9	41.9	25.5	1.1	68.6
B	10	73.9	44.5	26.5	0.8	71.8
303	10	76.3	47.6	26.3	0.9	75.0

Derris extract produces a physiological effect upon the flies distinctly different from that of pyrethrum, aliphatic thiocyanate, or pine oil (alone). It is much slower in manifesting its toxicity than any of these other materials. Tables 17 and 18 show distinctly that significant mortality occurred beyond the 24-hour period. This characteristic of derris has been reported by Campbell, et. al. (1934). The flies "knockeddown" by derris and by pyrethrum are readily distinguishable. With pyrethrum a majority lie upon their backs, apparently dead; with derris, only a few are entirely disabled, and the amjority assume a "semi-upright" position and display feeble, imperfectly coordinated movements with their appendages.

Available commercial derris extracts and pyrethrum extracts do not possess the same degree of compatibility with petroleum

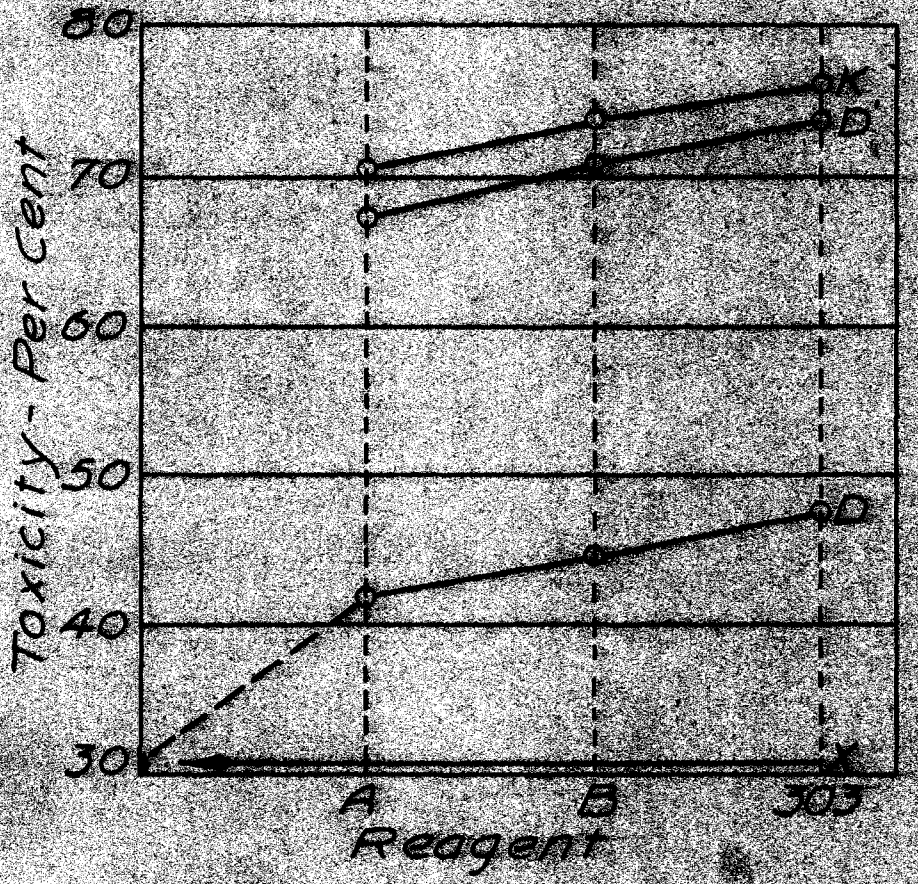

FIG. 15. ACTIVATION OF DERRIS (1-1/2% DERRIS-PINE OIL EXTRACT) WITH SEVERAL REAGENTS (10%) IN BASE OIL NO. 40 (K=DOWN IN 10 MINUTES; D=DEAD IN 24 HOURS; D^1=DEAD IN 48 HOURS; I=PROBABLE TOXICITY OF EXTRACT ALONE)

oils. A 1 per cent concentration of the derris-pine oil extract produced a noticeable cloudiness when incorporated with base oil No. 40, less cloudiness with No. 40 plus 4 per cent No. 303, and a clear solution with 14 and 24 per cents No. 303 in No. 40. Apparently this cloudiness may be reduced by either of two methods: (1) a reduction in the concentration of extract, or (2) an increase in the amount of pine oil added. If a high kill is to be maintained, the latter procedure is preferable.

Rotenone

Activation.

In order to obtain data for a comparison of the ability of pine oil No. 303 to activate derris extract and rotenone, a series of tests were conducted, using formulae containing rotenone (2 per cent concentrate) and rotenone plus several concentrations of No. 303. The results obtained from this series are summarized in Table 21. Figure 16 presents curves for the "knockdown", and 24- and 48-hour mortality records. By comparison of results, it may be seen that No. 303 pine oil activates rotenone (e.p.) at a rate similar to its effect upon derris extract. Rotenone was found to be similar to derris extract both as to speed of action (toxicity) and the appearance of the flies affected.

TABLE 21. SUMMARY OF THE TOXICITY OF ROTENONE (2% ROTENONE-PINE OIL CONCENTRATE) WITH PINE OIL NO. 303 IN BASE OIL NO. 40 TO THREE-DAY-OLD FLIES.

Per Cent Pine Oil	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead 24 Hours	24-48 Hours	48-72 Hours	72 Hour Total
2	9	38.5	27.0	7.0	0.4	34.5
5	9	45.6	31.7	9.6	0.1	41.5
15	9	66.5	47.5	14.5	0.6	62.7
25	9	85.3	67.8	14.6	0.5	82.9

Aliphatic Thiocyanate

Activation.

Inasmuch as the combination of pine oil No. 303 with pyrethrum, derris, and rotenone gave such favorable results, it seemed possible that it might produce similar effects when combined with an aliphatic thiocyanate. Tests with 3 per cent and 2 per cent of this material were conducted according to the same procedure followed in the other activation tests. Tables 22 and 23 summarize the results from these series. The 3 per cent concentration gave 100 per cent "knockdown" in all tests, The 2 per cent concentration also showed a high "knockdown"

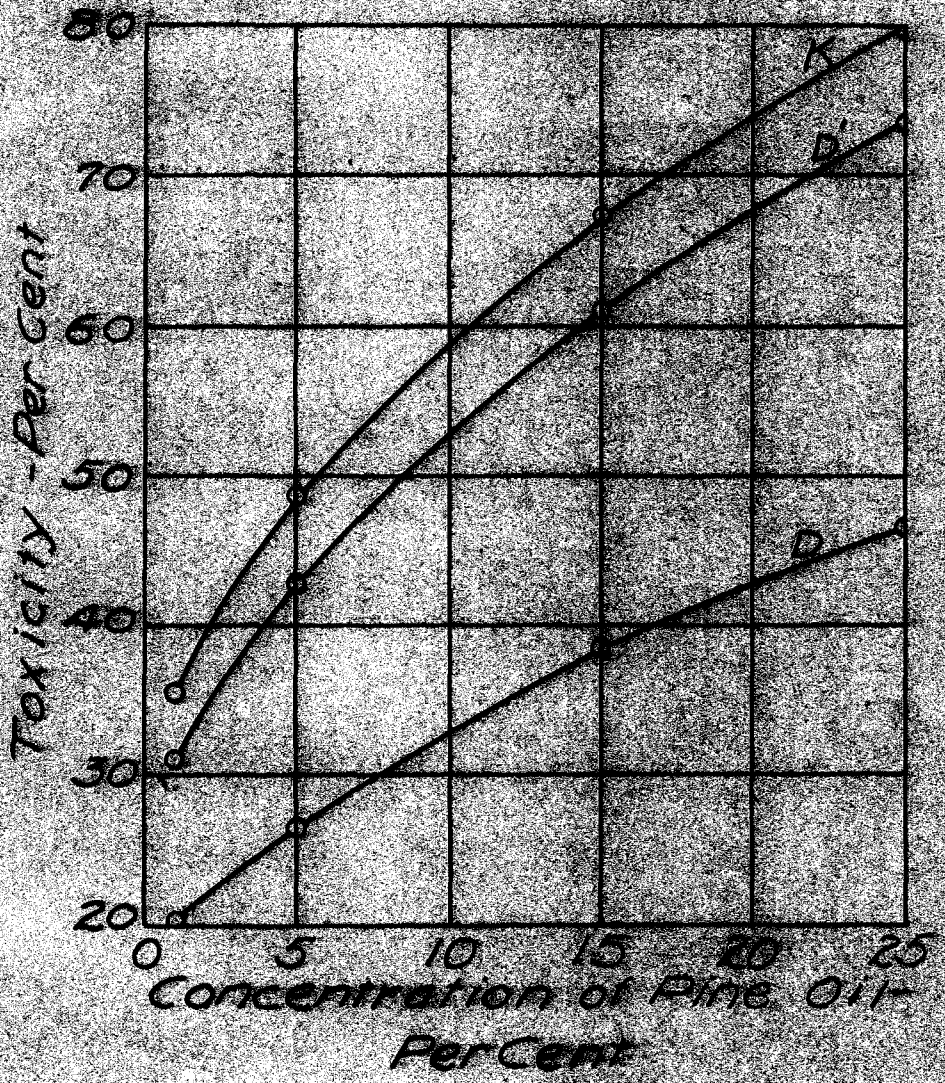

Fig. 16. ACTIVATION OF ROTENONE (PINE OIL CONCENTRATE) WITH PINE OIL NO. 303 IN BASE OIL NO. 40. (K=DOWN IN 10 MINUTES; D=DEAD IN 24 HOURS; D^1=DEAD IN 48 HOURS)

TABLE 22. SUMMARY OF THE TOXICITY OF 3 PER CENT ALIPHATIC THIOCYANATE WITH PINE OIL NO. 303 IN BASE OIL NO. 40 TO THREE-DAY-OLD FLIES.

Per Cent Pine Oil	Number of Tests	Per Cent Dead			
		24 Hours	24-48 Hours	48-72 Hours	72 Hour Total
0	8	24.4	2.4	1.2	28.1
5	8	24.7	2.3	0.9	27.9
15	8	27.2	5.1	1.9	34.4
25	8	25.8	5.4	1.6	32.9

TABLE 23. SUMMARY OF THE TOXICITY OF 2 PER CENT ALIPHATIC THIOCYANATE WITH PINE OIL NO. 303 IN BASE OIL NO. 40 TO THREE-DAY-OLD FLIES.

Per Cent Pine Oil	Number of Tests	Per Cent Down in 10 Minutes	Per Cent Dead in 24 Hours
0	8	99.5	4.9
5	8	100	3.1
15	8	100	3.1
25	8	100	3.3

(99+%), but produced a very insignificant mortality. The addition of pine oil No. 303, in concentrations as high as 25 per cent, produced no apparent activation with either concentration of the aliphatic thiocyanate. Due to these unfavorable results, no further activation tests were conducted. The rate of mortality from this toxic agent is similar to that of pyrethrum, its entire effect being manifested within 24 hours after exposure. Its initial effect, the "knockdown", occurs most rapidly.

REPELLENCE TESTS

Five series of field tests were completed during each of the summers of 1934 and 1935. Series I, 1934, was conducted with a privately-owned (J. M. Mendinhall) herd near New Castle, Delaware, and the remaining series with the herd at the University of Delaware Experimental Farm, Newark. Only Holstein-Friesians were sprayed in each herd. Base oil No. 40 was incorporated in all of the formulae tested for repellence. Air temperature data were recorded during all tests and relative humidity records were included during the summer of 1935. A summary of information concerning all ten of these repellence tests is given in Table 24.

The stable fly, Stomoxys calcitrans (L.), was the predominating species observed. Occasionally the house fly, Musca domestica L., equalled the stable fly in numbers, but was generally less abundant. The horn fly, Haematobia irritans (L.), appeared in small numbers and was not consistent throughout the summer in its attacks.

Methods. Series I, II, and III, 1934, were conducted according to the method given by Pearson, Wilson and Richardson (1933). An improvement in procedure, already reported by the writer (1935), was employed for the remaining series. The procedure in question consists of hourly observations for

TABLE 24. OUTLINE OF REPELLENCE TESTS, 1934-35

Series	Type of Sprayer	Treatment During Preliminary Period	Number of Cows	Objective
1934				
I	Hand	No Spray	15	Repellence of pine oil
II	Electric	" "	15	" " " "
III	"	" "	20	" " " "
IV	"	Base Oil	15	Repellence of pyrethrum and pine oil
V	"	" "	15	Repellence of pyrethrum and pine oil
1935				
I	Electric	Base Oil	20	Repellence of pyrethrum
II	"	" "	20	Repellence of derris
III	"	" "	20	Repellence of derris and pine oil
IV	"	" "	20	Repellence of aliphatic thiocyanate
V	"	" "	20	Repellence of aliphatic thiocyanate and pine oil

eight consecutive days on cows staked individually. On the day preceding a series of tests, all of the cows are thoroughly washed. The first four days constitute a preliminary period, during which the cows are sprayed with a base oil (alone) at 6:00 A.M. daily, and hourly fly counts are made. From the average of these counts the cows are assigned to groups of five each, on a basis of their individual fly susceptibility. During the following four days the cows are sprayed daily, as just described, with the same base oil, in which has been incorporated the repellent ingredient or ingredients under test. Fly counts are made as before. With the older method, no spray was applied during the preliminary period. Two tables are given to illustrate the records obtained by this procedure: Table 25 is an example of the individual fly counts for one day during a preliminary period; Table 26 demonstrates the assignment of the cows to groups on a basis of the individual preliminary period averages.

Equipment. The materials in Series I, 1934, were applied with hand sprayers, but in all subsequent tests an electric sprayer (Plate 5) was employed. The latter was found to be much more satisfactory. Pint fruit jars (individually graduated) served as containers, a separate one being used for each formula. Each cow was treated with approximately 60 c.c. of spray material, this amount being delivered by the electric sprayer in less than one minute.

TABLE 25. INDIVIDUAL FLY COUNTS FOR ONE DAY DURING A PRELIMINARY PERIOD; ALL COWS SPRAYED WITH NO. 40 BASE OIL (DATA FOR THE FIRST DAY OF PRELIMINARY PERIOD COUNTS IN SERIES V, 1934).

Cow	Hour 7	8	9	10	11	12	1	2	3	Total	Average
1	2	13	18	37	38	16	17	48	26	215	23.9
2	2	7	15	21	20	16	21	20	27	149	16.5
3	2	15	16	28	40	23	30	53	22	229	25.4
4	2	18	40	49	40	30	42	32	39	292	32.4
5	6	13	10	8	38	29	25	25	17	171	19.0
6	2	9	13	26	47	40	48	26	18	229	25.4
7	4	10	18	35	39	25	38	29	44	242	26.9
8	3	28	33	30	63	54	46	47	56	360	40.0
9	2	59	58	70	85	95	70	46	61	546	60.6
10	1	16	30	23	41	32	24	35	30	232	25.7
11	3	9	24	24	28	40	35	45	17	225	25.0
12	5	57	47	69	53	32	42	71	75	431	47.9
13	4	11	10	10	27	15	12	8	22	119	13.2
14	1	6	22	35	30	32	22	48	75	271	30.1
15	4	9	16	27	37	12	35	38	45	223	24.7
Total	45	260	370	492	626	491	507	571	574	3934	437.1
Average	2.8	17.3	24.6	32.8	41.7	32.7	33.8	38.0	38.2	262.2	29.1
Air Temperature °F	69	75	76	78	80	82	80	82	81	703	78.1

TABLE 26. SUMMARY OF FLY COUNTS OBTAINED DURING A PRELIMINARY PERIOD, WITH THE COWS GROUPED FOR SPRAYING (DATA FOR THE PRELIMINARY PERIOD DURING SERIES V,1934)

Spray Material	Cow	Average Number of Flies Per Count				4-Day Total	Average	Group Average
		*9/15/34	9/17/34	9/18/34	9/19/34			
	7	26.9	43.6	23.0	33.7	127.2	31.8	
1 lb. Pyrethrum	10	25.7	18.3	13.7	33.9	91.6	22.9	
per gallon	11	25.0	16.6	9.9	23.3	74.8	18.7	27.4
in No. 40	12	47.9	20.6	16.0	49.3	133.8	33.4	
	15	24.7	28.6	32.0	36.4	121.7	30.4	
3/4 lb. Pyrethrum	1	23.9	19.3	19.1	28.1	90.4	22.6	
per gallon	2	16.5	11.9	23.7	31.3	83.4	20.8	
10% No. 303	3	25.4	26.1	17.4	47.4	116.3	29.1	27.5
in No. 40	5	19.0	38.5	21.9	47.0	126.4	31.6	
	9	60.6	19.2	23.7	29.9	133.4	33.3	
1/2 lb. Pyrethrum	4	32.4	22.4	25.9	48.5	129.2	32.3	
per gallon,	6	25.4	29.2	17.0	44.4	116.0	29.0	
15% No. 303	8	40.0	29.0	17.0	27.2	113.2	28.3	27.5
in No. 40	13	13.8	21.1	19.3	20.1	73.7	18.4	
	14	30.1	25.7	25.0	37.2	118.0	29.5	

*Data from Table 25.

An iron peg, leather halter, and 6 to 8 feet of rope (Plate 6) served to stake each cow individually. The leather halters, bearing an identification tag on each side, were left on the cows throughout an entire series. The tether ropes were permanently attached to the iron pegs, and lead ropes were employed for transferring the cows from stanchions to pasture. The cows were staked near each other, but sufficiently far apart to prevent contact (Plate 7). The pegs were moved to fresh ground each day.

Pine Oil

Series I, 1934. A series to determine the repellence of pine oil. The formulae selected for comparison were: (1) No. 40 base oil alone, and (2) 10 per cent No. 303 pine oil in No. 40. A "No Spray" group was included for comparison. Preliminary counts were made for two days, and were followed by five days of spraying. Table 27 summarizes all counts in the series and Figure 17 presents a summary of the air temperature records and preliminary period fly trend. The relative repellence of the formulae is summarized in Figure 18. Both of the sprays were superior to "No Spray", but No. 40 base oil alone showed greater efficiency than 10 per cent No. 303 in No. 40.

Plate 5. Electric Sprayer in Operation

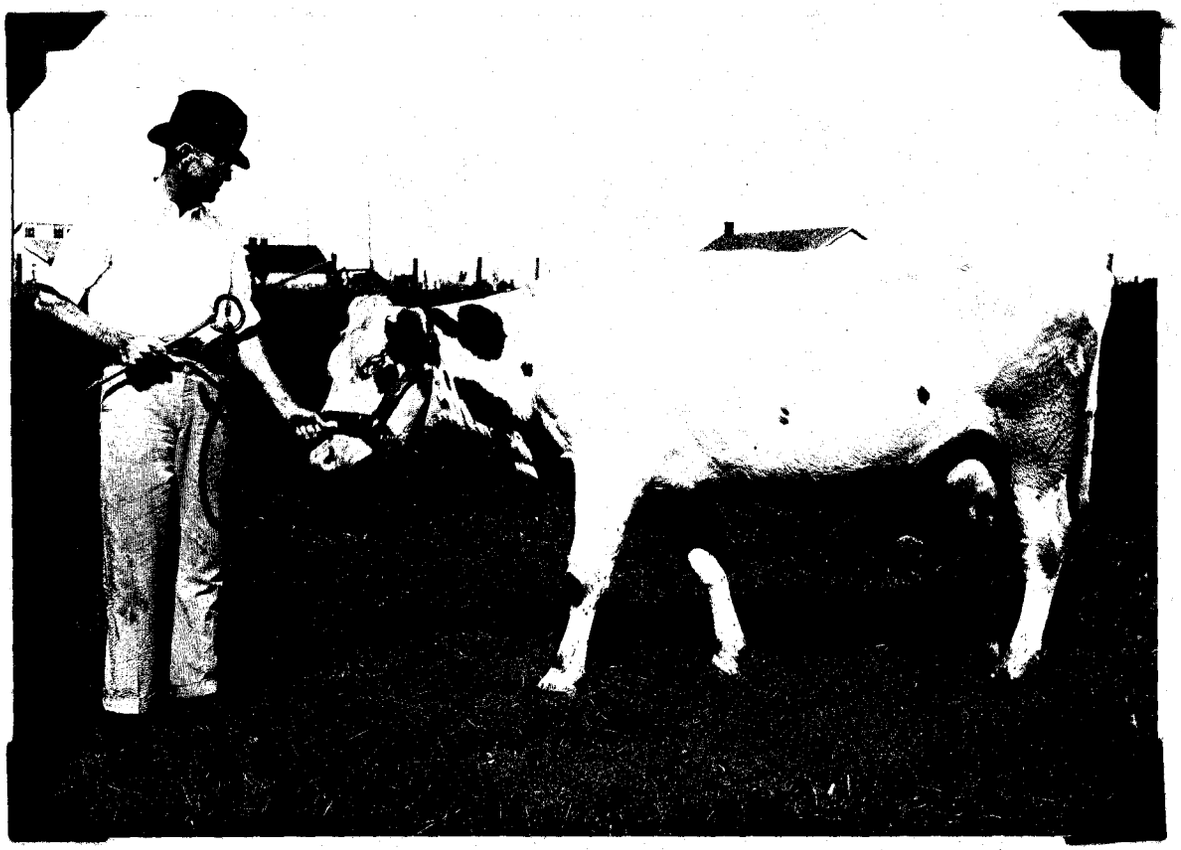

Plate 6. Equipment Used for Staking the Cows: Leather Halter, Rope and Iron Peg.

Plate 7. Cows Staked Individually for Fly Counts.

TABLE 27. SUMMARY OF THE FLY COUNTS DURING SERIES I, 1934.

Spray Formula	Average Number of Flies per Cow per Count: Preliminary Period 2 Days	Spraying Period 5 Days
No Spray	55.3	47.2
No. 40 Base Oil	55.9	26.3
10% No. 303 in No. 40	56.4	32.1

Series II, 1934. A second series to determine the repellence of pine oil. Due to the unexpected results of series I, 1934, it was decided to duplicate it, but with a preliminary period of four days and a spraying period of like duration. Table 28 summarizes the fly counts and Figure 19 presents an average of the temperature records and the preliminary period fly trend. Figure 20 presents the average fly populations on the sprayed cows. From the results of this and the preceding series, it was evident that 10 per cent pine oil No. 303 possessed insufficient repellent properties to merit its use as the sole added repellent in an oil-base cattle fly spray.

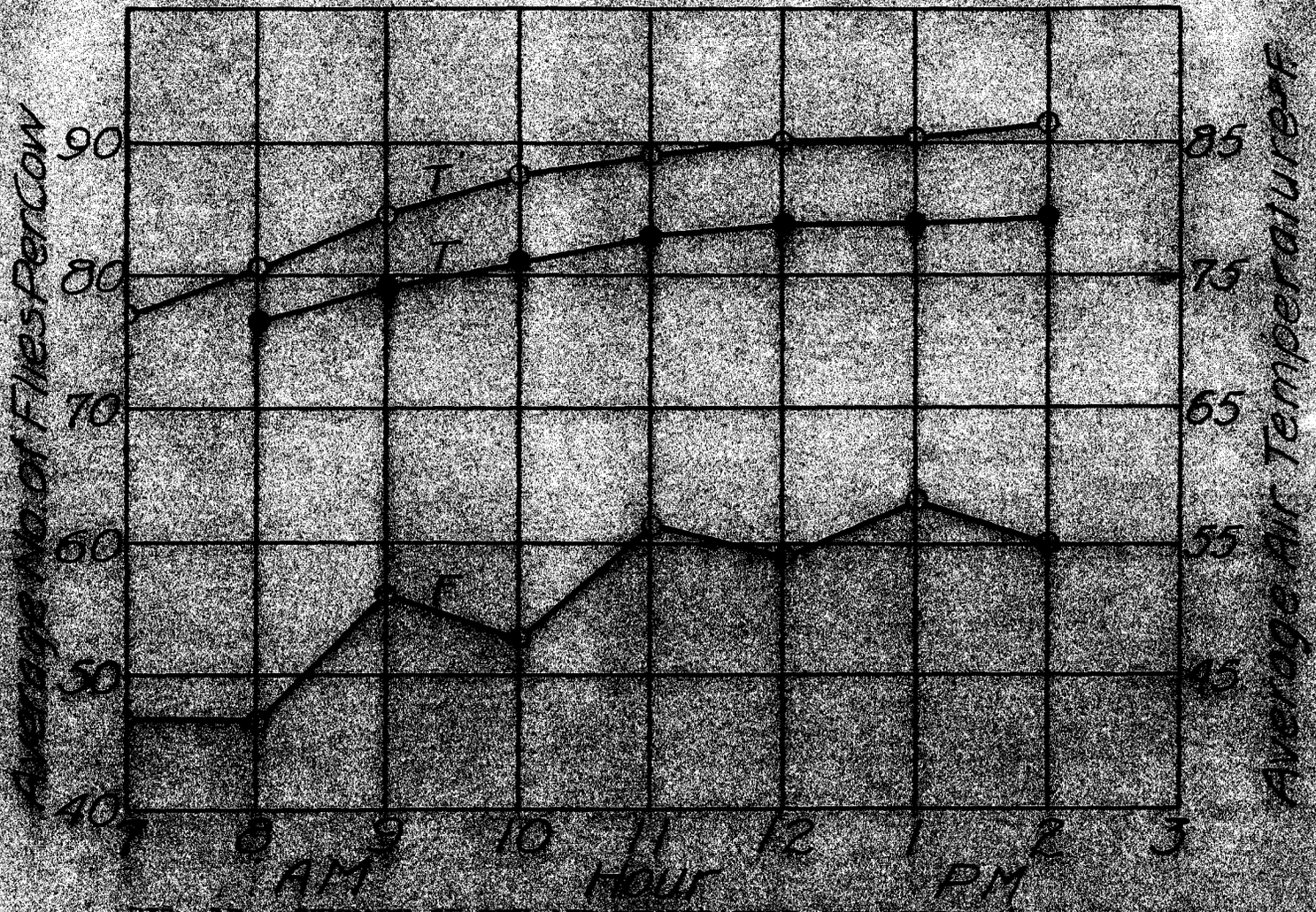

FIG. 11. SUMMARY OF THE AIR TEMPERATURE AND PRELIMINARY PERIOD FLY TREND DATA FOR SERIES 1, 1954 (F=PRELIMINARY PERIOD FLY TREND; T=AVERAGE AIR TEMPERATURE DURING PRELIMINARY PERIOD; T^1=AVERAGE AIR TEMPERATURE DURING SPRAYING PERIOD)

FIG. 18. SUMMARY OF THE FLY POPULATION DURING THE SPRAYING PERIOD IN SERIES I, 1934 (A=NO SPRAY; B=10 PER CENT NG. 303 IN NO. 40; C=NO. 40 ALONE)

TABLE 28. SUMMARY OF THE FLY COUNTS DURING SERIES II, 1934.

Spray Formula	Average Number of Flies per Cow per Count: Preliminary Period 4 Days	Spraying Period 4 Days
No Spray	46.5	37.4
No. 40 Base Oil	46.8	21.0
10% No. 303 in No. 40	46.4	19.6

Series III, 1934. A third series to determine the repellence of pine oil. Since 10 per cent No. 303 failed to produce a noticeable increase in the repellence of No. 40, it seemed desirable to investigate the merits of higher concentrations of the same material. Concentrations of 10, 15, and 25 per cent No. 303 in No. 40 were tested and, for comparative purposes, a 1 pound of pyrethrum per gallon formula was also included. Table 29 is a summary of the fly counts. Figure 21 presents a summary of the temperature records and the preliminary period fly trend. The average temperature records for the preliminary period and the spraying period were so nearly identical that it seemed impractical to present them separately. Figure 22 gives a comparison of the four formulae.

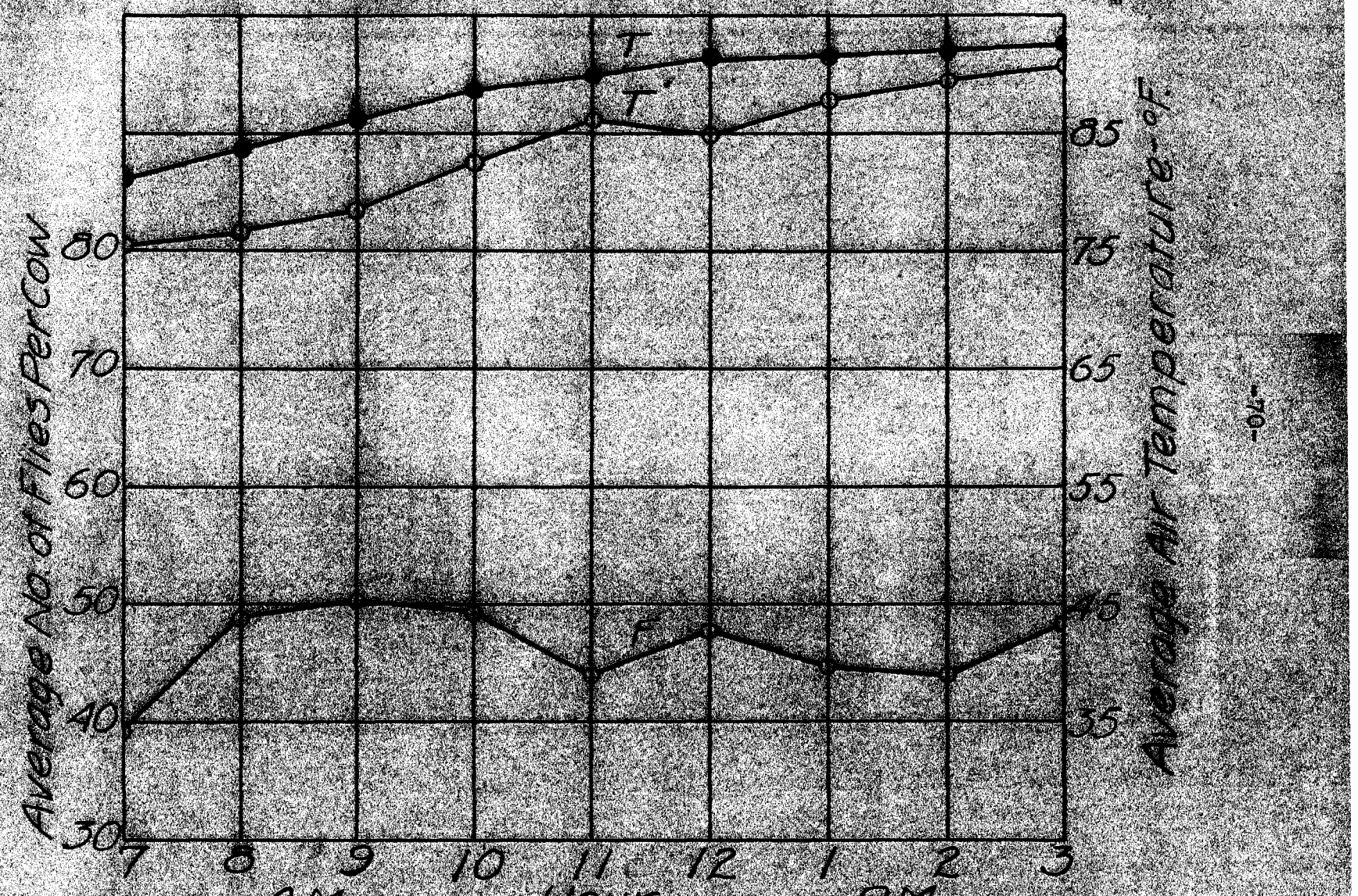

FIG. 19. SUMMARY OF THE AIR TEMPERATURE AND PRELIMINARY PERIOD FLY TREND DATA FOR SERIES II, 1934 (P=PRELIMINARY PERIOD FLY TREND; T=AVERAGE TEMPERATURE DURING PRELIMINARY PERIOD; T^1=AVERAGE TEMPERATURE DURING SPRAYING PERIOD)

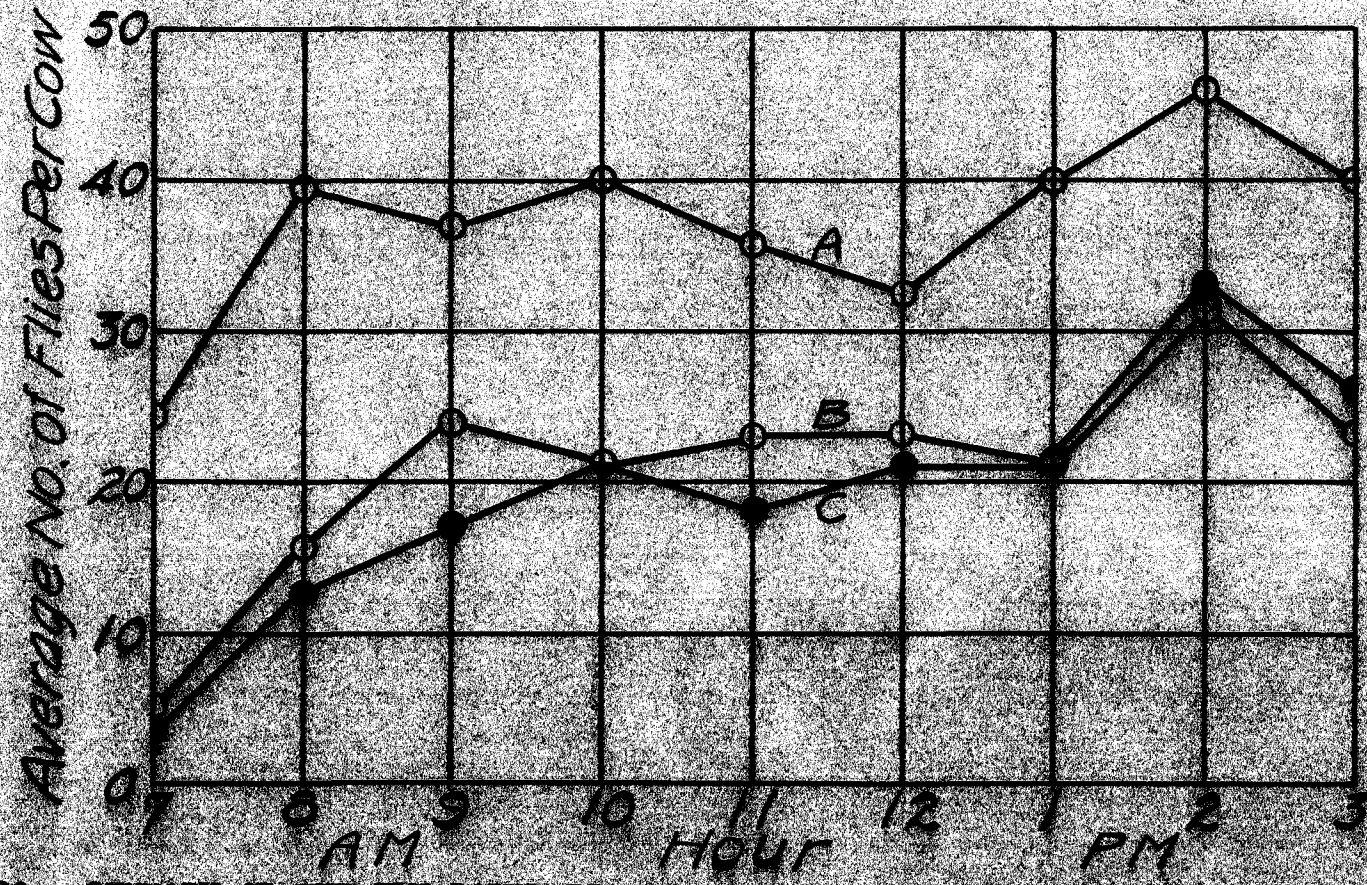

FIG. 20. SUMMARY OF THE FLY POPULATION DURING THE SPRAYING PERIOD IN SERIES II, 1934 (A=NO SPRAY; B=NO. 40 ALONE; C=10 PER CENT NO. 505 IN NO. 40)

TABLE 29. SUMMARY OF THE FLY COUNTS DURING SERIES III, 1934.

Spray Formula	Average Number of Flies per Cow per Count: Preliminary Period 4 Days	Spraying Period 4 Days
10% No. 303	43.6	15.6
15% No. 303	43.8	22.7
25% No. 303	44.3	16.4
1 lb. Pyrethrum	44.1	7.1

None of the concentrations of No. 303 compared favorably with the pyrethrum (alone) formula.

Pyrethrum and Pine Oil

Series IV, 1934. A series to determine the repellence of pyrethrum and pine oil. The results of the first three series indicated that the use of pine oil No. 303 (alone) in base oil No. 40, as a repellent, could not be justified. A similar conclusion had already been drawn from toxicity data. Three formulae were next tested to determine the ability of No. 303 to activate pyrethrum as a repellent. The formulae were: 1/2 pound pyrethrum plus 15 per cent No. 303, 3/4 pound pyrethrum plus 10 per cent No. 303, and 1 lb. pyrethrum (alone). Table 30 summarizes the fly counts for the entire series and Figure

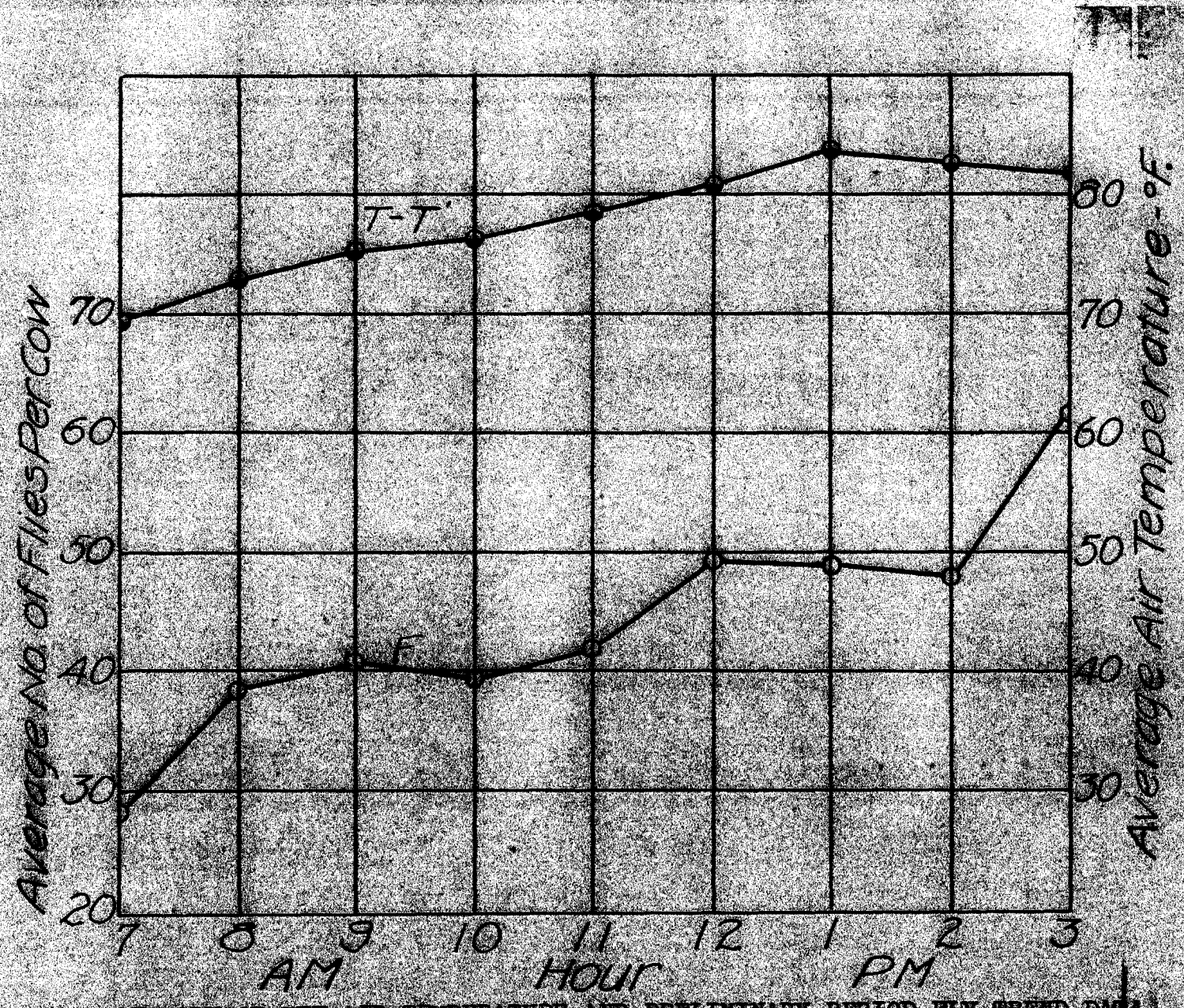

FIG. 21. SUMMARY OF THE AIR TEMPERATURE AND PRELIMINARY PERIOD FLY TREND DATA FOR SERIES III, 1954 (F=PRELIMINARY PERIOD FLY TREND; $T-T^1$=AVERAGE AIR TEMPERATURE - IDENTICAL - DURING THE PRELIMINARY AND SPRAYING PERIODS)

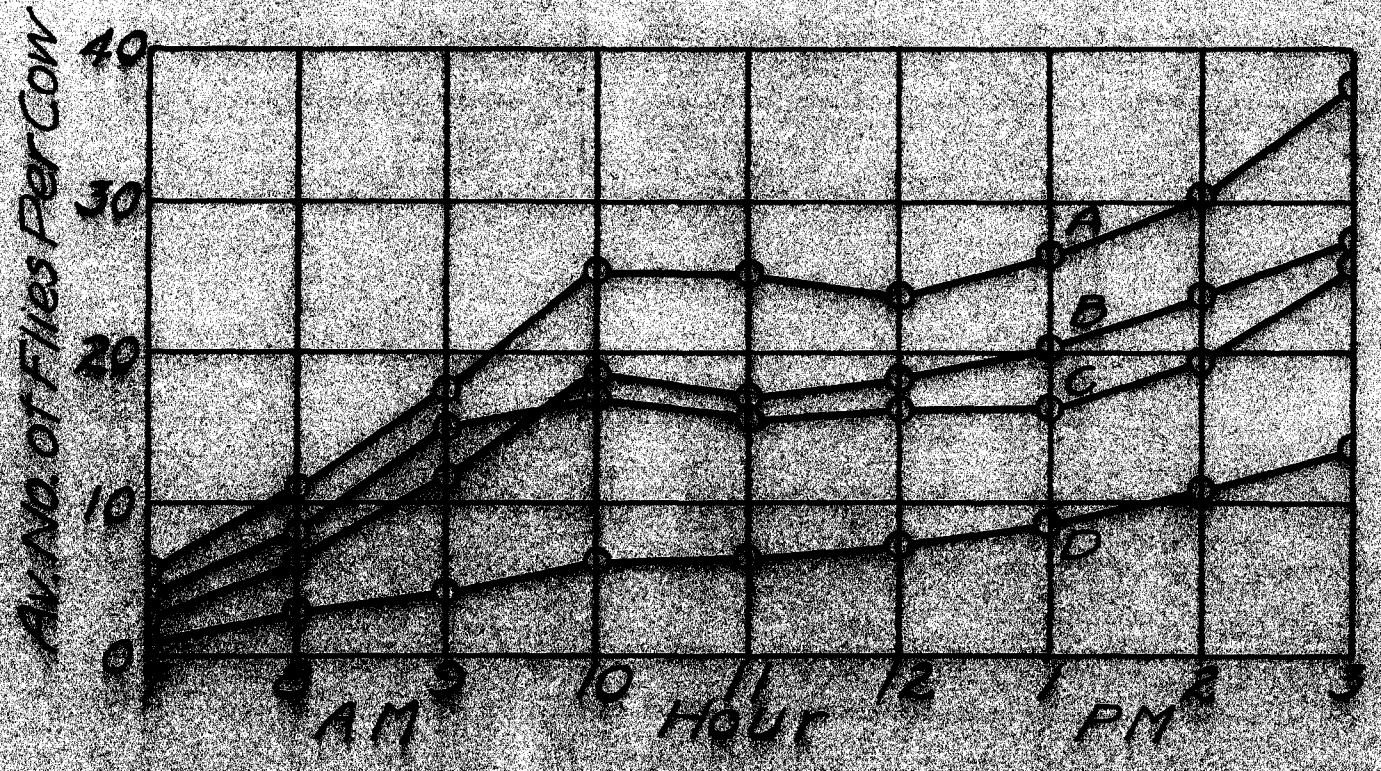

FIG. 22. SUMMARY OF THE FLY POPULATION DURING THE SPRAYING PERIOD IN SERIES III, 1934 (A=15 PER CENT NO. 303 IN NO. 40; B=25 PER CENT NO. 303 IN NO. 40; C=10 PER CENT NO. 303 IN NO. 40; D=1 POUND OF PYRETHRUM PER GALLON IN NO. 40)

TABLE 30. SUMMARY OF THE FLY COUNTS DURING SERIES IV, 1934.

Spray Formula	Average Number of Flies per Cow per Count: Preliminary Period* 4 Days	Spraying Period 4 Days
1 lb. Pyrethrum	26.0	7.2
3/4 lb. Pyrethrum, 10% No. 303	26.1	7.8
1/2 lb. Pyrethrum, 15% No. 303	26.2	9.8

*All cows sprayed daily during preliminary period with No. 40 base oil.

23 presents a summary of the air temperature and preliminary period fly trend data. In this, and the remaining series, in which pine oil alone was not tested, the relative repellence data are not presented on an hourly trend basis, but the average of the fly population for each formulae is given instead. The fly counts during the spraying period in this series have been combined with those in Series V, 1934, and are presented in Figure 24.

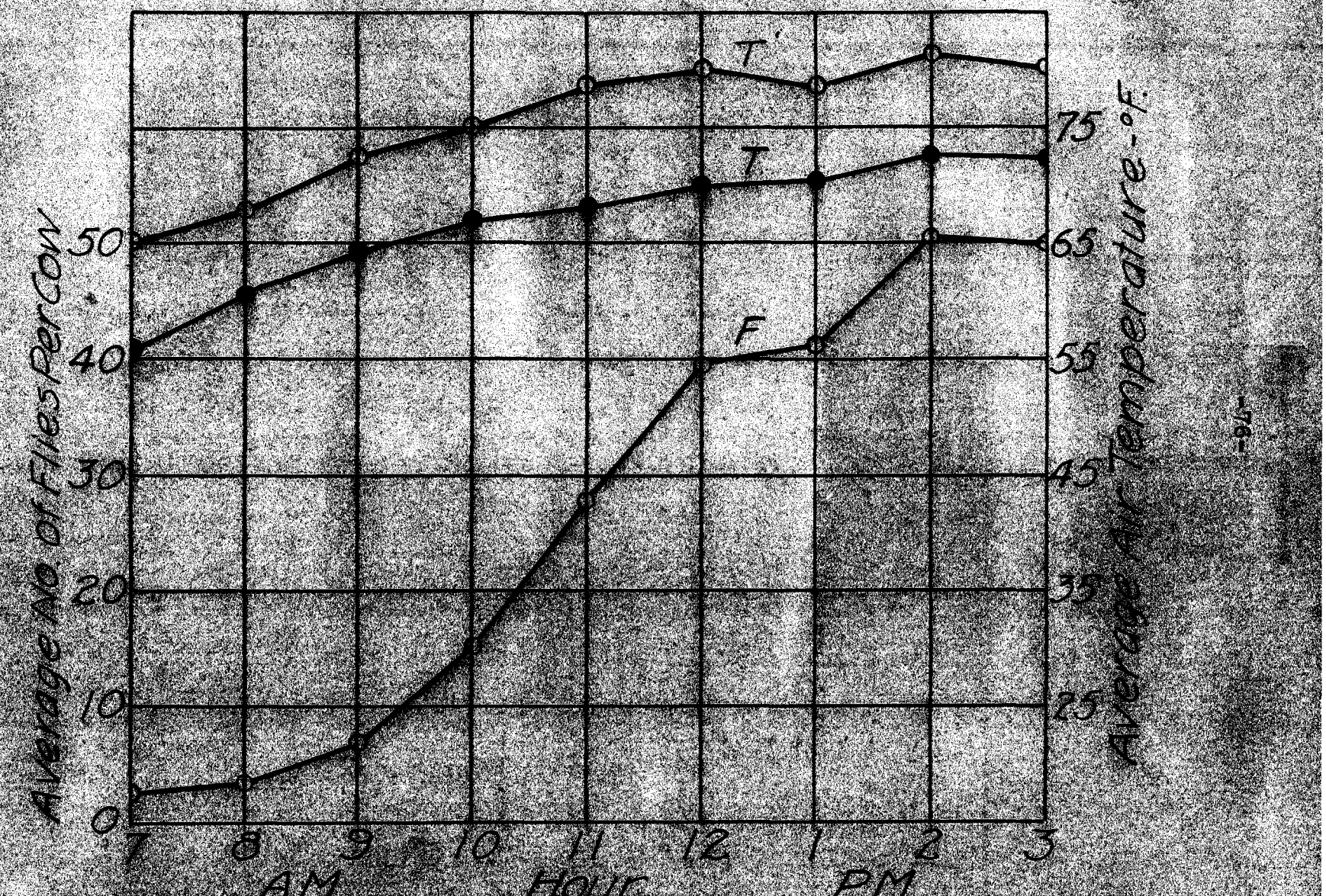

FIG. 23. SUMMARY OF THE AIR TEMPERATURE AND PRELIMINARY PERIOD FLY TREND DATA FOR SERIES IV, 1934 (P=PRELIMINARY PERIOD FLY TREND; T=AVERAGE AIR TEMPERATURE DURING PRELIMINARY PERIOD; T^1=AVERAGE AIR TEMPERATURE DURING SPRAYING PERIOD)

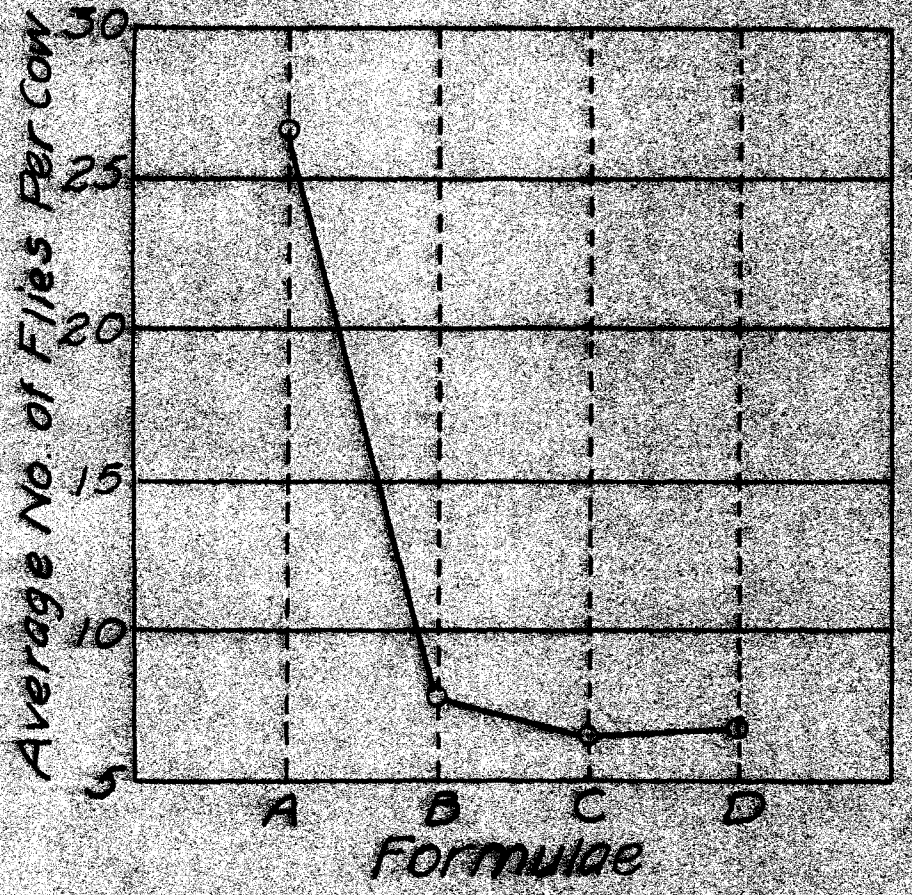

FIG. 24. SUMMARY OF FLY COUNTS DURING SERIES IV, AND V, 1934, COMBINED (FORMULAE: A=PRELIMINARY COUNTS; B=1/2 POUND PYRETHRUM PLUS 15 PER CENT NO. 303; C=3/4 POUND PYRETHRUM PLUS 10 PER CENT NO. 303; D=1 POUND PYRETHRUM)

Series V, 1934. A second series to determine the repellence of pyrethrum and pine oil. The formulae given under Series IV, 1934, were again tested for the purpose of securing further repellence data with them. Table 31 summarizes the fly counts and Figure 25 presents an average of the air temperature and preliminary period fly trend data. The relative repellence data are referred to under Series IV, 1934. It is apparent from the results of both series that there is no marked difference in the efficiency of the three formulae as fly repellents.

Pyrethrum

Series I, 1935. A series to determine the relative repellence of several concentrations of pyrethrum. Concentrations of 1/4, 1/2, 3/4, and 1 pound per gallon were selected for investigation. Table 32 summarizes all of the counts; Figure 26 presents the average temperature and humidity data for the series, and also the preliminary period fly trend. Figure 27 presents the repellence curve obtained with the four formulae. Due to unusually cool weather during early summer, a relatively low fly population was present to attack the cows. However, a consistent relationship was found between repellence and the several concentrations of pyrethrum. With a larger fly population it is to be expected that the higher concentrations would show greater relative efficiency.

TABLE 31. SUMMARY OF THE FLY COUNTS DURING SERIES V, 1934.

Spray Formula	Average Number of Flies per Cow per Count: Preliminary Period* 4 Days	Spraying Period 4 Days
1 lb. Pyrethrum	27.4	6.6
3/4 lb.Pyrethrum, 10% No. 303	27.5	5.5
1/2 lb.Pyrethrum, 15% No. 303	27.5	5.8

*All cows sprayed daily during preliminary period with No. 40 base oil.

TABLE 32. SUMMARY OF THE FLY COUNTS DURING SERIES I, 1935.

Spray Formula	Average Number of Flies per Cow per Count: Preliminary Period* 4 Days	Spraying Period 4 Days
1/4 lb.Pyrethrum	8.4	3.2
1/2 lb.Pyrethrum	8.3	2.6
3/4 lb.Pyrethrum	8.3	2.1
1 lb. Pyrethrum	8.3	1.7

*All cows sprayed daily during preliminary period with No. 40 base oil.

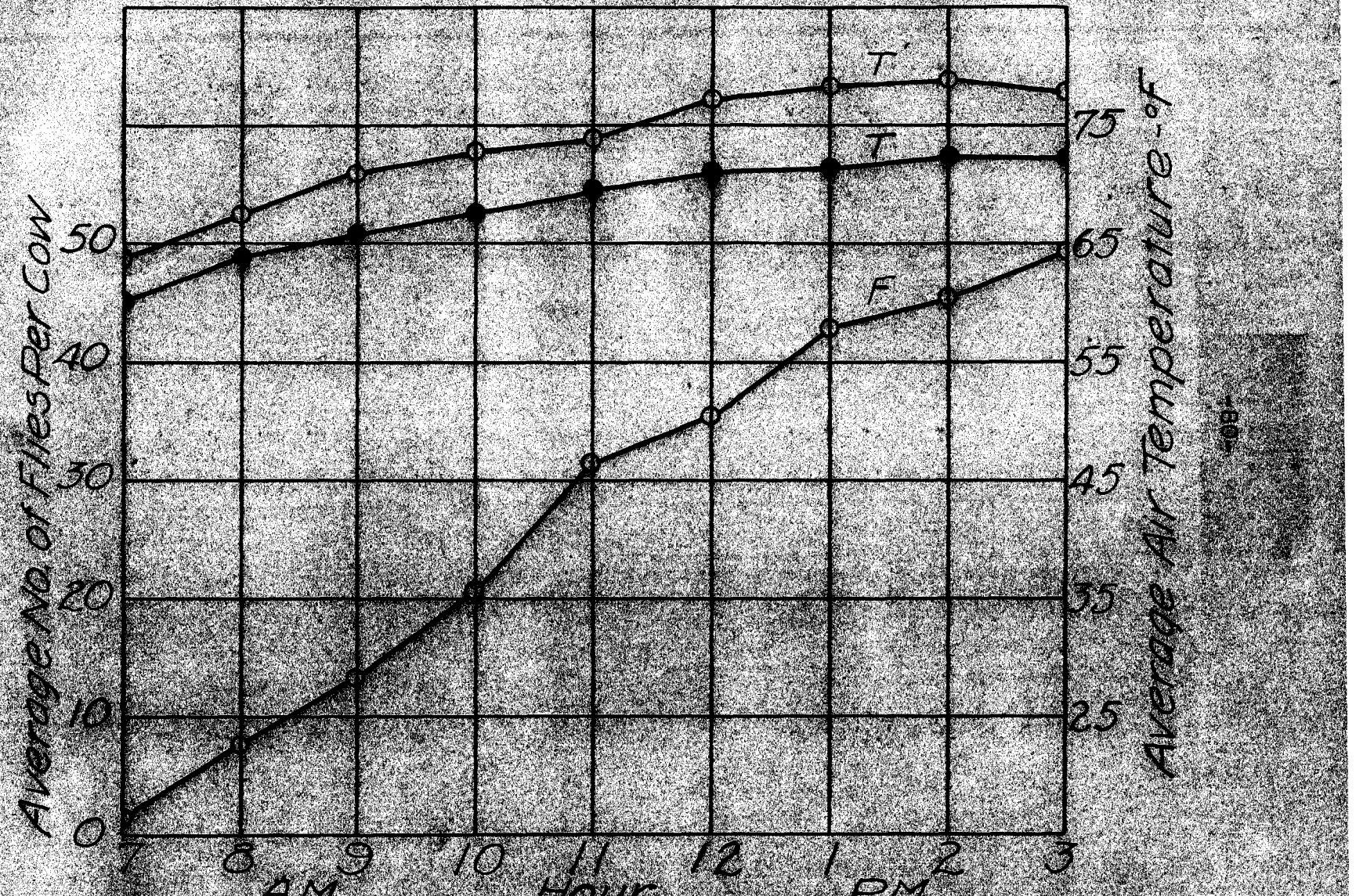

FIG. 25. SUMMARY OF THE AIR TEMPERATURE AND PRELIMINARY PERIOD FLY TREND DATA FOR SERIES Y, 1934 (F=PRELIMINARY PERIOD FLY TREND; T=AVERAGE AIR TEMPERATURE DURING PRELIMINARY PERIOD; T_1=AVERAGE AIR TEMPERATURE DURING SPRAYING PERIOD)

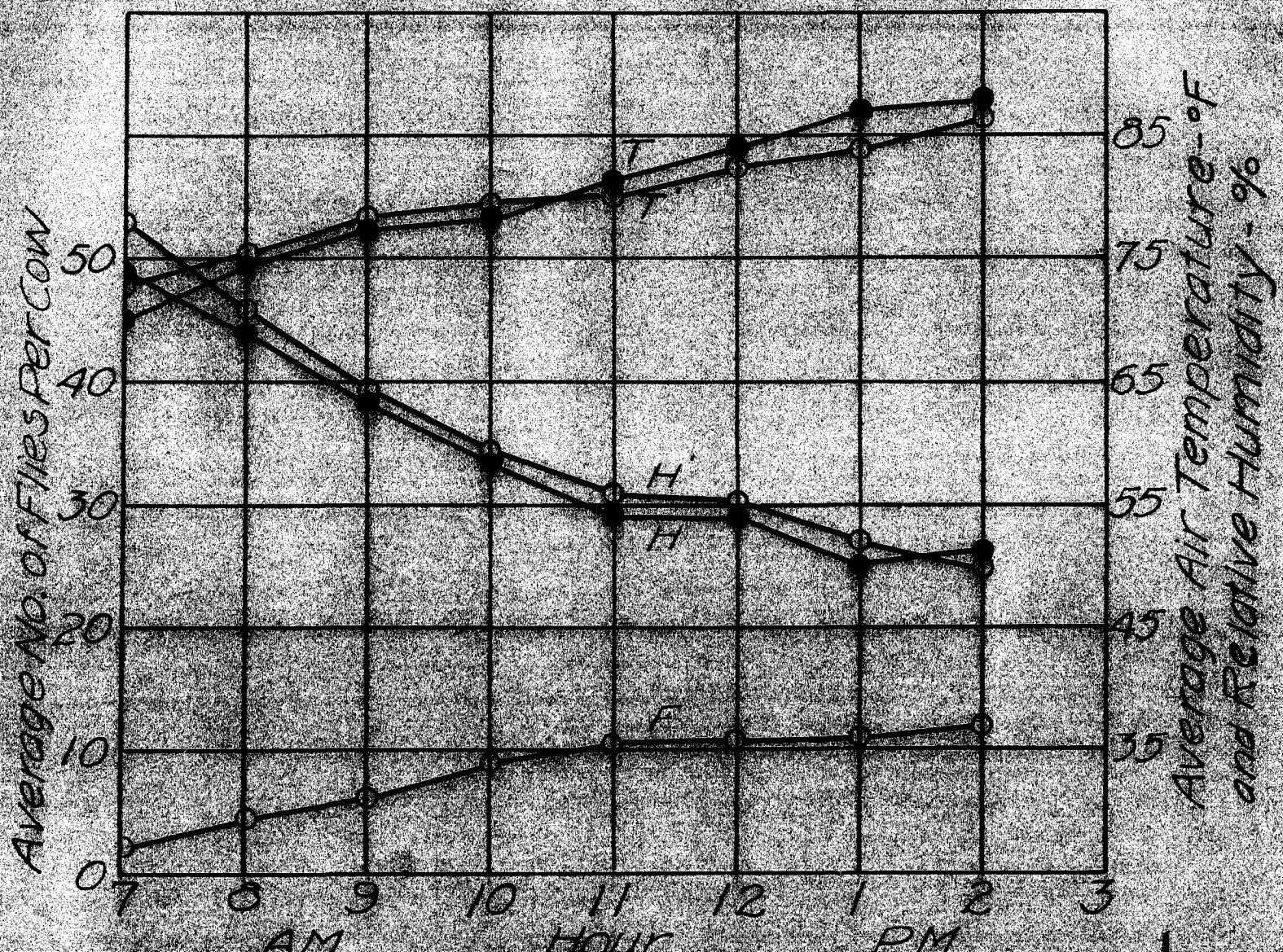

FIG. 26. SUMMARY OF THE AIR TEMPERATURE, RELATIVE HUMIDITY, AND PRELIMINARY PERIOD FLY TREND DATA FOR SERIES I, 1935 (F=PRELIMINARY PERIOD FLY TREND; H=RELATIVE HUMIDITY DURING PRELIMINARY PERIOD; H^1=RELATIVE HUMIDITY DURING

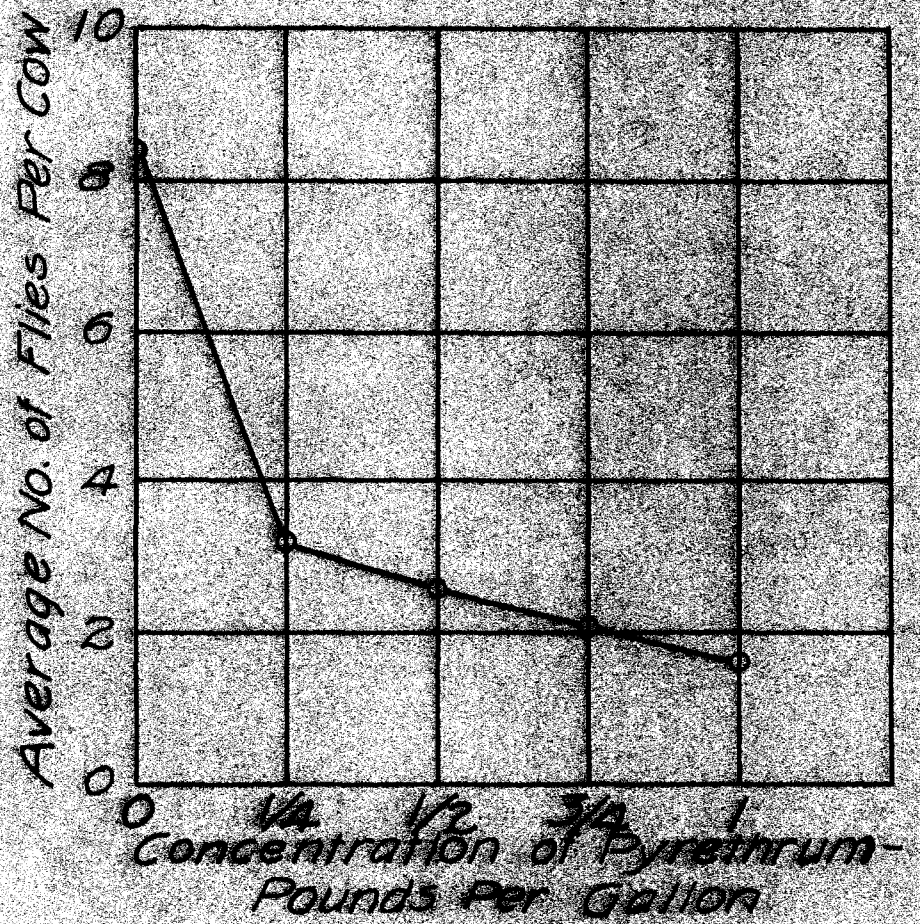

FIG. 27. SUMMARY OF FLY COUNTS DURING SERIES I, 1935
(O CONCENTRATION = PRELIMINARY COUNTS)

Derris

Series II, 1935. A series to determine the relative repellency of several concentrations of derris extract. Concentrations of 0.5, 1.0, 1.5, and 2.0 per cent extract were tested. In Table 33 is given a summary of the fly counts and in Figure 28, the average of the temperature, humidity, and preliminary period fly trend data. Figure 29 presents the curve obtained from the fly counts during the spraying period. The data in Table 33 indicate that a smaller fly population was attacking the cows during the preliminary period than during the spraying period. For example, the group sprayed with 0.5 per cent derris extract showed an average fly population greater than that recorded during the preliminary period. This sudden increase in the number of flies was probably due to the emergence of a new brood of adults. Such a condition makes it difficult to compare the formulae with base oil (alone), but apparently it did not disturb the relative susceptibility of the individual cows, since the results of the several formulae show a consistent relationship to each other.

TABLE 33. SUMMARY OF THE FLY COUNTS DURING SERIES II, 1935.

Spray Formula	Average Number of Flies per Cow per Count: Preliminary Period* 4 Days	Spraying Period 4 Days
0.5% Derris Extract	13.7	15.9
1.0% Derris Extract	13.6	12.2
1.5% Derris Extract	13.6	10.8
2.0% Derris Extract	13.6	9.2

*All cows sprayed daily during preliminary period with No. 40 base oil.

Derris and Pine Oil

Series III, 1935. A series to determine the repellence of derris and pine oil. The following formulae were tested: 1.0 per cent derris extract (alone), 1.0 per cent derris extract plus 5 per cent No. 303, 1.0 per cent derris extract plus 15 per cent No. 303, and 2.0 per cent derris extract (alone). Table 34 is a summary of the fly counts. Figure 30 presents an average of the temperature and relative humidity data for the entire series, and also the fly trend during the preliminary period. Figure 31 presents a summary

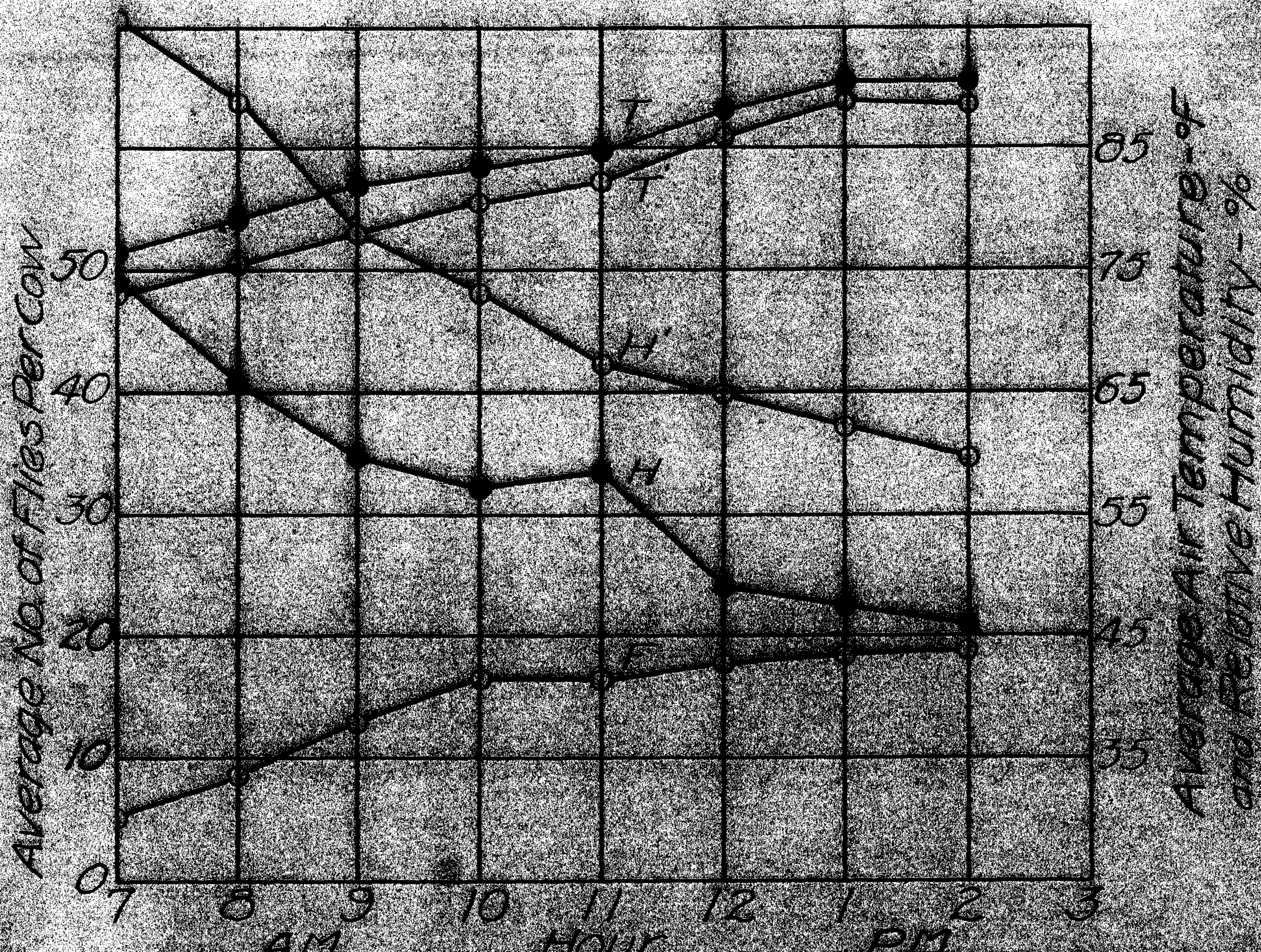

FIG. 28. SUMMARY OF THE AIR TEMPERATURE, RELATIVE HUMIDITY, AND PRELIMINARY PERIOD FLY TREND DATA FOR SERIES II, 1935 (F=PRELIMINARY PERIOD FLY TREND; H=RELATIVE HUMIDITY DURING PRELIMINARY PERIOD; H[1]=RELATIVE HUMIDITY DURING

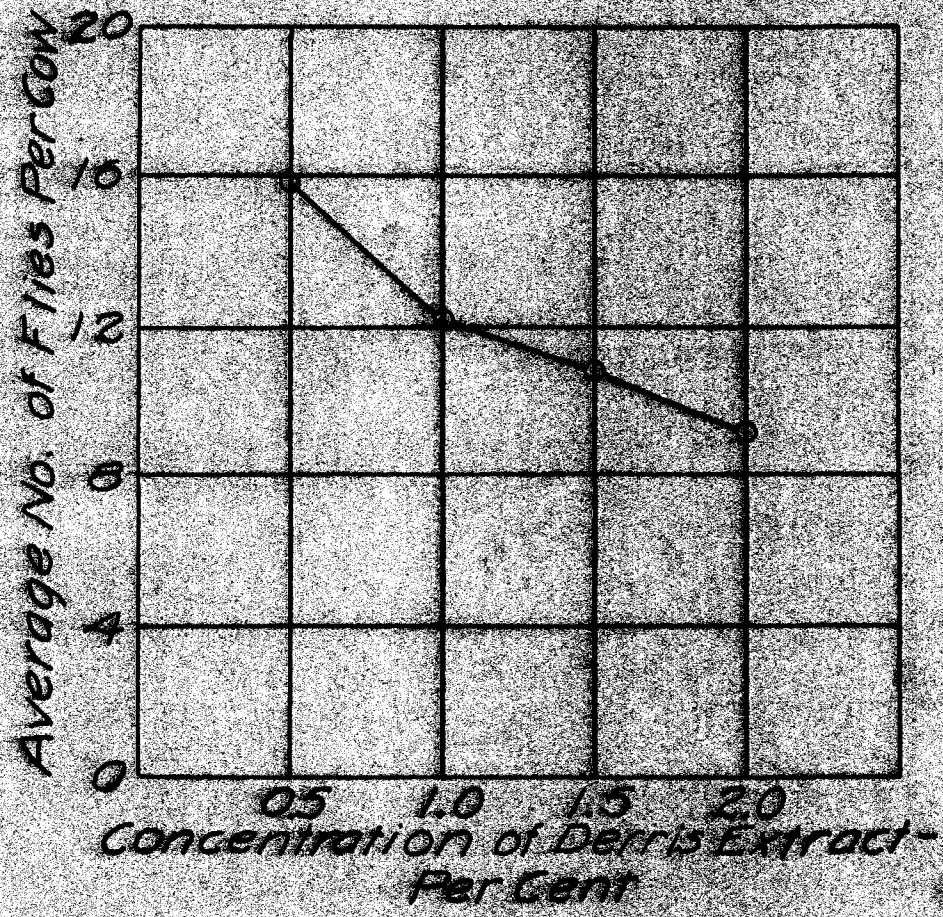

FIG. 29. SUMMARY OF FLY COUNTS FOR THE SPRAYING PERIOD DURING SERIES II, 1935

TABLE 34. SUMMARY OF THE FLY COUNTS DURING SERIES III, 1935.

Spray Formula	Average Number of Flies per Cow per Count: Preliminary Period* 4 Days	Spraying Period 4 Days
1% Derris Extract	25.4	17.7
1% Derris Extract 5% No. 303	25.3	16.9
1% Derris Extract 15% No. 303	25.3	16.0
2% Derris Extract	25.3	14.1

*All cows sprayed daily during preliminary period with No. 40 base oil.

of the results for the four formulae. The addition of 5 and 15 per cents, respectively, of pine oil No. 303 caused a slight increase in the repellence of 1.0 per cent derris extract. Neither of these combinations were equal to 2.0 per cent of the extract (alone).

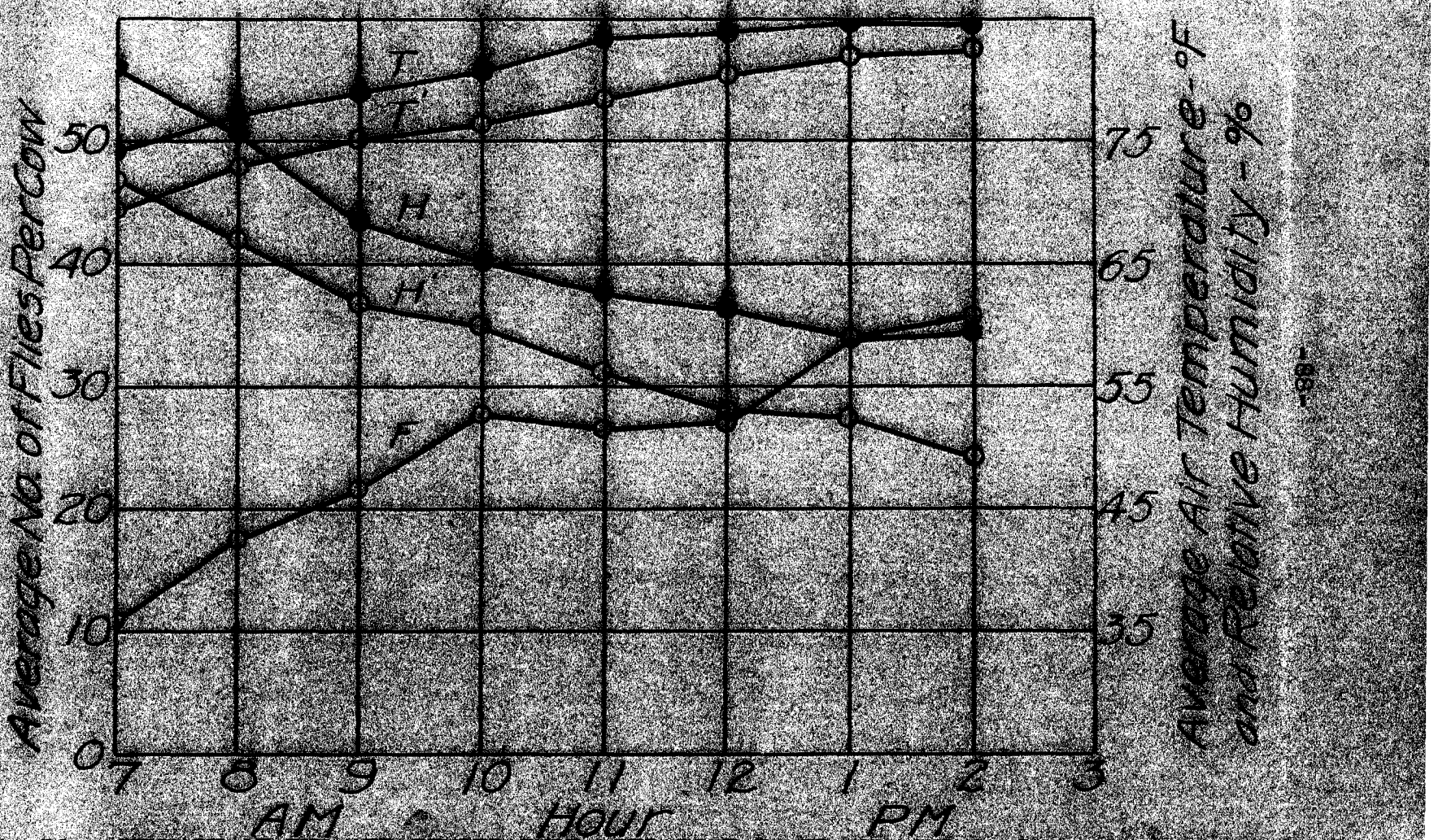

FIG. 30. SUMMARY OF THE AIR TEMPERATURE, RELATIVE HUMIDITY, AND PRELIMINARY FLY TREND DATA FOR SERIES III, 1955 (F=PRELIMINARY PERIOD FLY TREND; H=RELATIVE HUMIDITY DURING PRELIMINARY PERIOD; H^1=RELATIVE HUMIDITY DURING SPRAYING PERIOD; T=TEMPERATURE DURING PRELIMINARY PERIOD; T^1=TEMPERATURE DURING SPRAYING PERIOD)

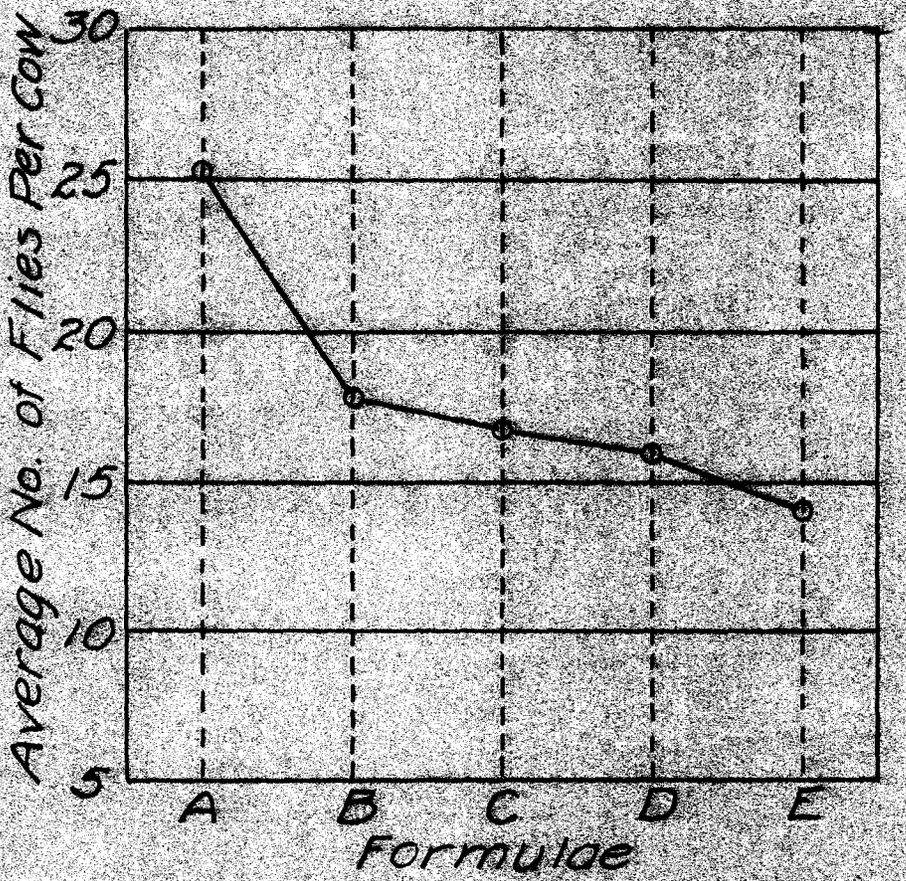

FIG. 31. SUMMARY OF FLY COUNTS DURING SERIES III, 1935. (FORMULAE: A=PRELIMINARY COUNTS; B=1 PER CENT DERRIS EXTRACT; C=1 PER CENT DERRIS EXTRACT PLUS 5 PER CENT NO. 303; D=1 PER CENT DERRIS EXTRACT PLUS 15 PER CENT NO. 303; E=2 PER CENT DERRIS EXTRACT)

Aliphatic Thiocyanate

Series IV, 1935. A series to determine the relative repellence of several concentrations of aliphatic thiocyanate. Concentrations of 1, 2, and 3 per cent aliphatic thiocyanate (alone) were selected for testing and, for comparative purposes, a formula containing 1 pound of pyrethrum per gallon was also included. A summary of the fly counts is given in Table 35.

TABLE 35. SUMMARY OF THE FLY COUNTS DURING SERIES IV, 1935.

Spray Formula	Average Number of Flies per Cow per Count: Preliminary Period* 4 Days	Spraying Period 4 Days
1% Aliphatic Thiocyanate	23.7	16.9
2% Aliphatic Thiocyanate	23.7	15.9
3% Aliphatic Thiocyanate	23.7	14.9
1 lb. Pyrethrum	23.7	8.3

*All cows sprayed daily during preliminary period with No. 40 base oil.

Figure 32 presents a summary of the temperature, humidity, and preliminary period fly trend data. Figure 33 presents a summary of the results with these several formulae. All three of the concentrations of aliphatic thiocyanate were superior to base oil alone, but none of them compared favorably with the pyrethrum formula. Only a slight difference was found in the efficiency of the three aliphatic thiocyanate concentrations. During the first four hours of exposure these three formulae showed an efficiency in consistent relation with concentration, but for the last four observations, such was not the case. It seems probable, therefore, that after approximately four hours exposure, the aliphatic thiocyanate had undergone some change which rendered the several concentrations indistinctly different in efficiency.

Aliphatic Thiocyanate and Pine Oil

Series V, 1935. A series to determine the repellence of aliphatic thiocyanate and pine oil. The following formulae were tested: 1 per cent aliphatic thiocyanate plus 15 per cent No. 303, 2 per cent aliphatic thiocyanate plus 10 per cent No. 303, and 3 per cent aliphatic thiocyanate (alone). A formula containing 1/2 pound pyrethrum per gallon, plus 10 per cent No. 303 was included for comparison. The averages from all of the counts are given in Table 36. Figure 34 presents a summary of the temperature, humidity, and pre-

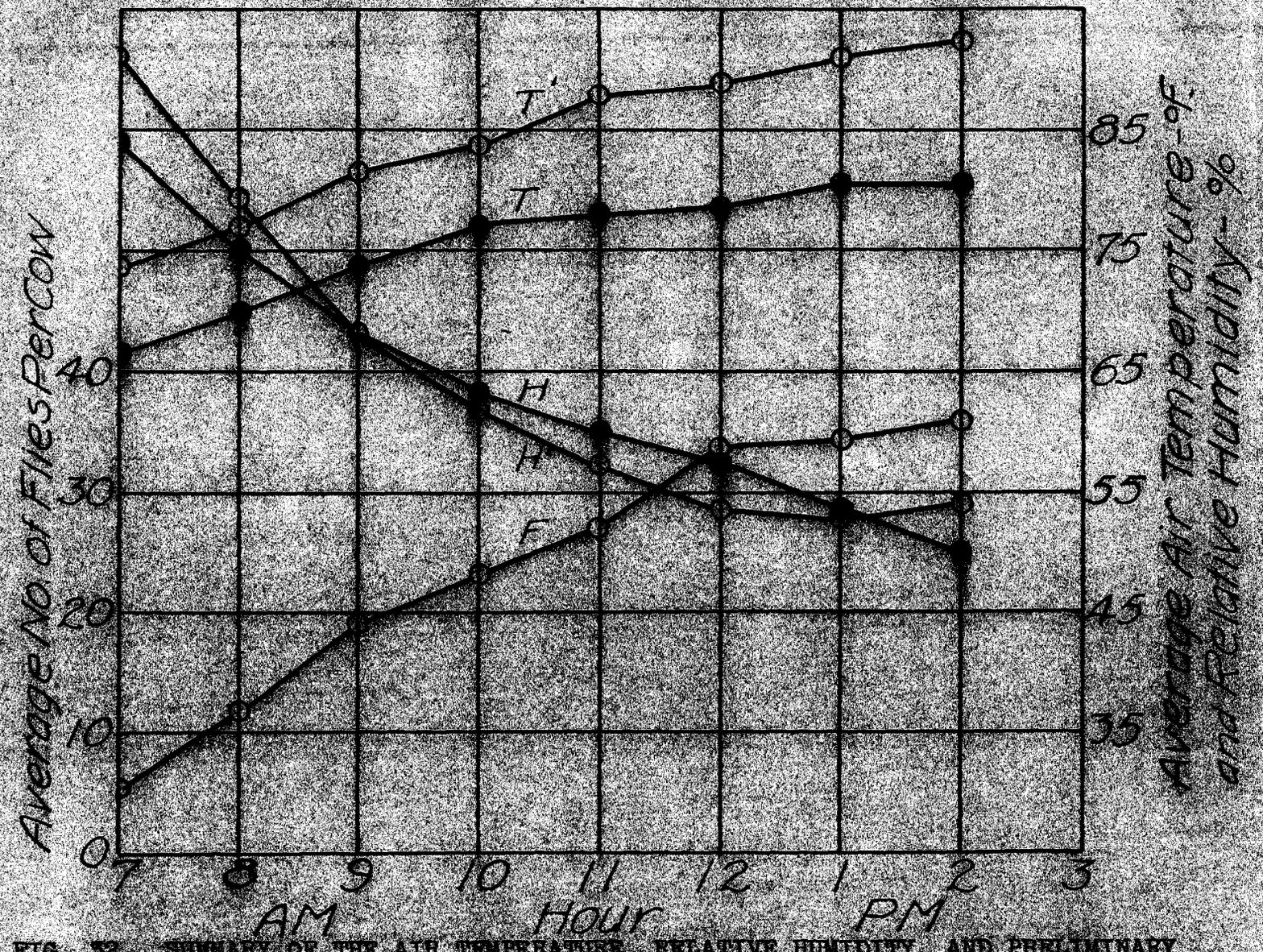

FIG. 52. SUMMARY OF THE AIR TEMPERATURE, RELATIVE HUMIDITY, AND PRELIMINARY PERIOD FLY TREND DATA FOR SERIES IV, 1955 (F=PRELIMINARY FLY TREND; H=HUMIDITY DURING PRELIMINARY PERIOD; H^1=HUMIDITY DURING SPRAYING PERIOD; T=TEMPERATURE DURING PRELIMINARY PERIOD; T^1=

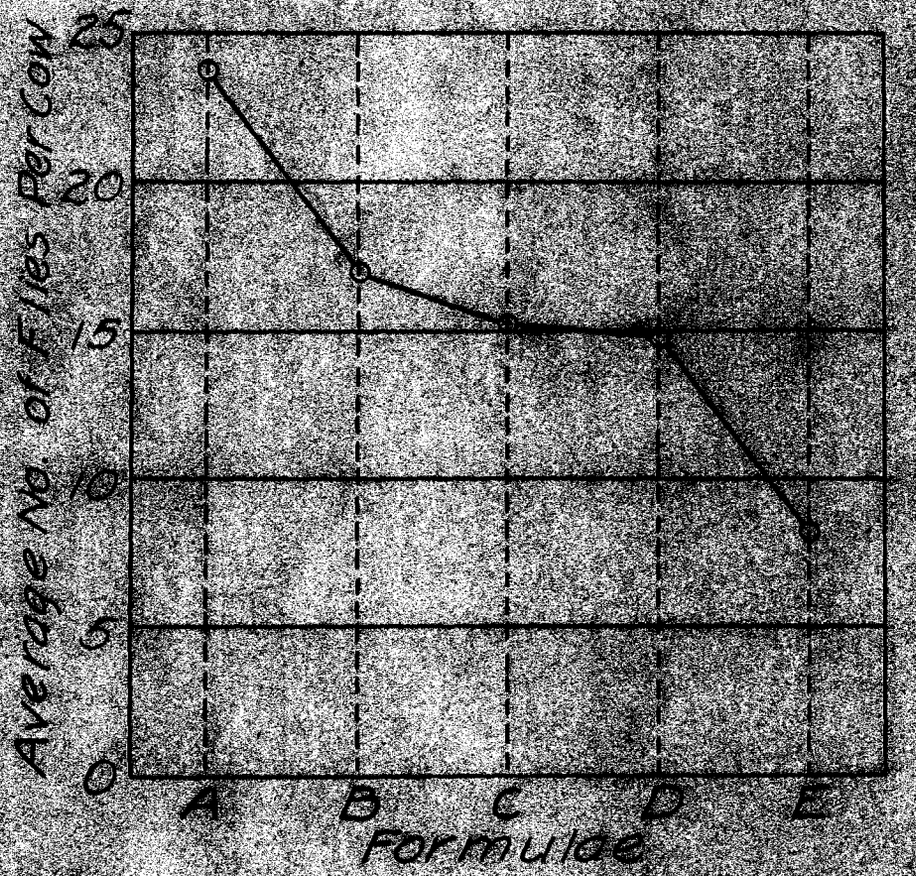

FIG. 33. SUMMARY OF FLY COUNTS DURING SERIES IV, 1935 (FORMULAE: A, PRELIMINARY COUNTS; B, C, and D, 1, 2 AND 3 PER CENT, RESPECTIVELY, ARTIFICIAL CHLOROPHANTE; E, 1 POUND PYRETHRUM PER GALLON)

liminary period fly trend data. The averages of the fly counts are presented in Figure 35. The three concentrations of aliphatic thiocyanate maintained a relationship similar to that found in Series IV, 1935. The addition of pine oil to the lower concentrations produced no apparent increase in repellence. The formula containing 1/2 pound pyrethrum plus 10 per cent No. 303 consistently showed greater repellence than any of the aliphatic thiocyanate formulae. The combination of aliphatic thiocyanate with pine oil No. 303 does not appear to be practical, either from the standpoint of toxicity or repellence.

TABLE 36. SUMMARY OF THE FLY COUNTS DURING SERIES V, 1935.

Spray Formula	Average Number of Flies per Cow per Count	
	Preliminary Period* 4 Days	Spraying Period 4 Days
1% Aliphatic Thiocyanate, 15% No. 303	22.6	14.2
2% Aliphatic Thiocyanate, 10% No. 303	22.6	11.8
3% Aliphatic Thiocyanate	22.6	11.7
1/2 lb. Pyrethrum 10% No. 303	22.6	8.6

*All cows sprayed daily during preliminary period with No. 40 base oil.

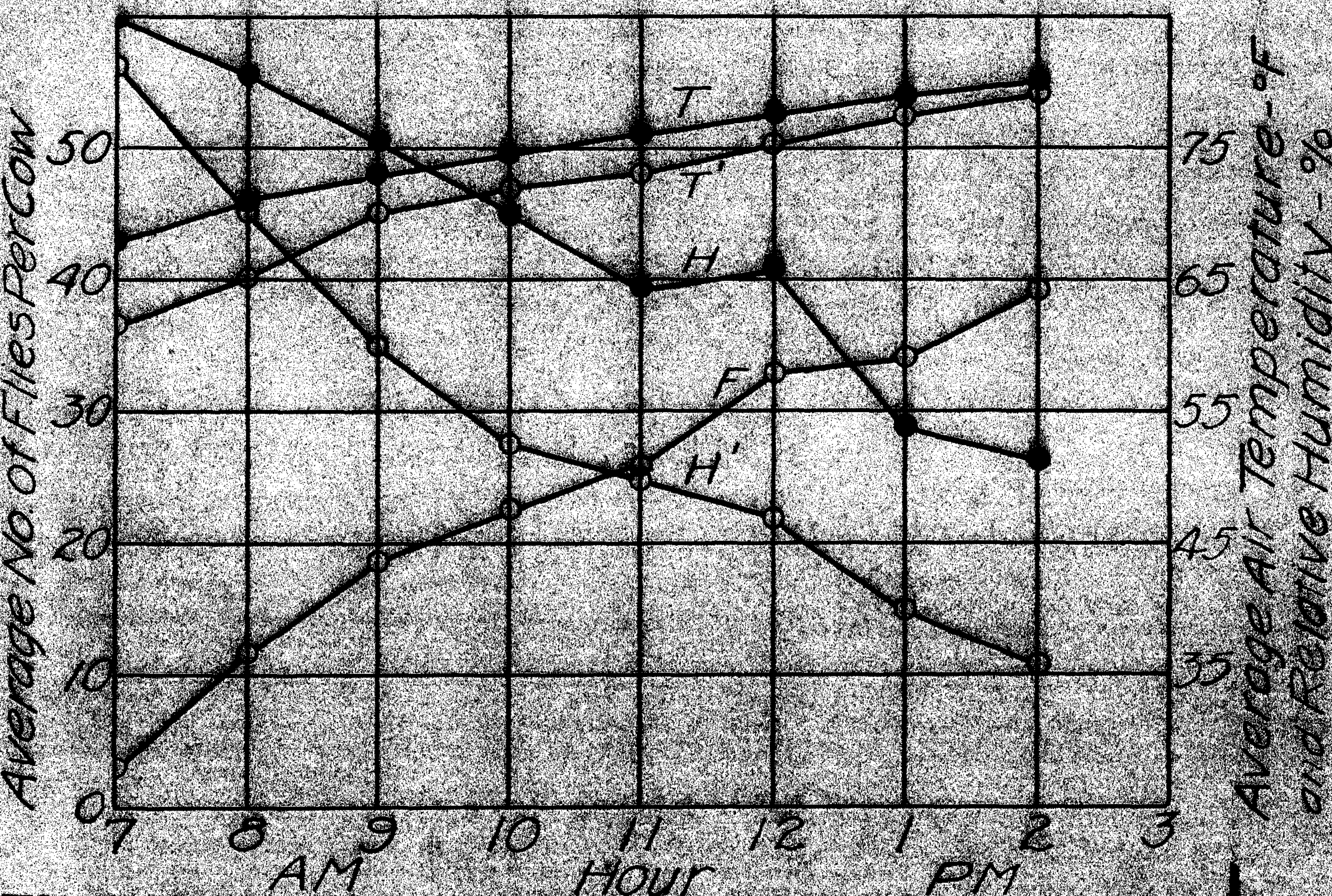

FIG. 34. SUMMARY OF THE AIR TEMPERATURE, RELATIVE HUMIDITY, AND PRELIMINARY PERIOD FLY TREND DATA FOR SERIES V, 1935 (F=PRELIMINARY PERIOD FLY TREND; H=HUMIDITY DURING PRELIMINARY PERIOD; H'=HUMIDITY DURING SPRAYING PERIOD; T=TEMPERATURE DURING PRELIMINARY PERIOD; T'= TEMPERATURE DURING SPRAYING PERIOD)

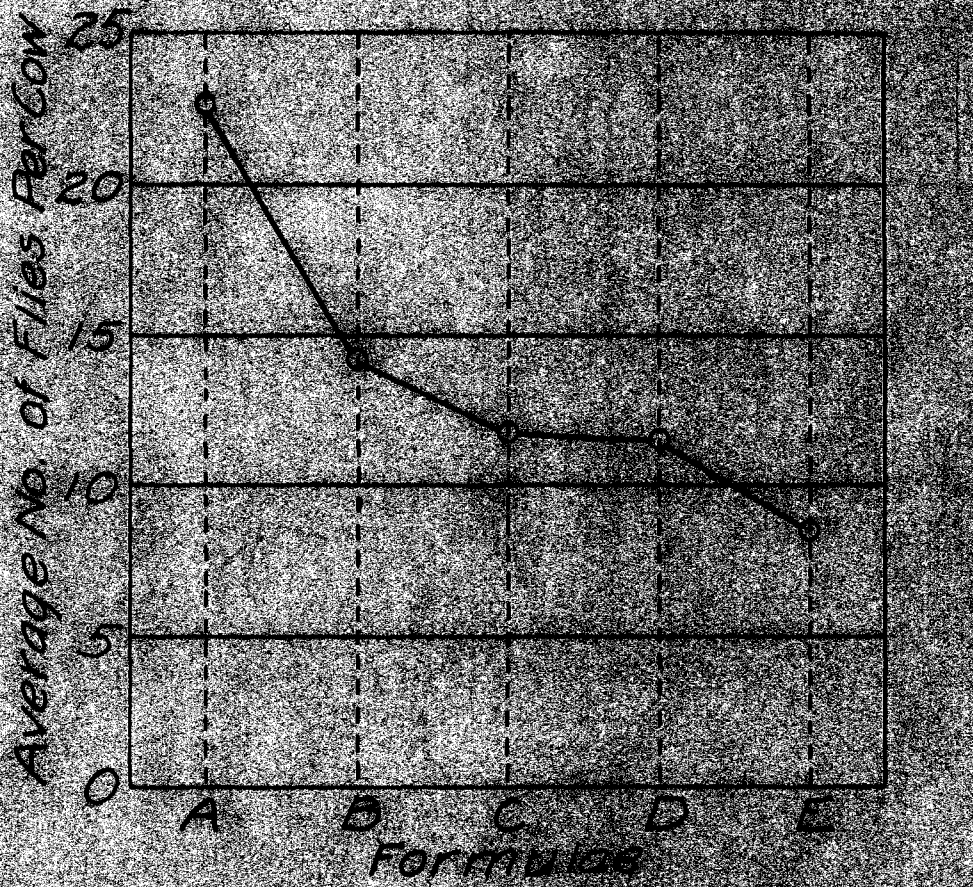

FIG. 35. SUMMARY OF FLY COUNTS DURING SERIES V, 1955 (FORMULAE: A-PRELIMINARY COUNTS; B-1 PER CENT ALIPHATIC THIOCYANATE PLUS 15 PER CENT NO. 363; C-2 PER CENT ALIPHATIC THIOCYANATE PLUS 10 PER CENT NO. 363; D-3 PER CENT ALIPHATIC THIOCYANATE; E-1/2 POUND PYRETHRUM PLUS 10 PER CENT NO. 363)

Fly Trend Observations

Field repellence tests depend entirely upon the attacks of "wild" flies to demonstrate the relative effectiveness of spray materials. If the flies characteristically attacked cows in greatest numbers at certain hours during the day, it would be advantageous to be able to predict such periods. Throughout the duration of the repellence tests reported herein, the daily fly counts were recorded graphically for the purpose of determining possible generalities applicable to the hourly fly trend. It was found that the flies did not consistently follow any definite hourly trend for each day throughout an entire season. During relatively hot days (above 90° F.) they may show such a trend as is presented in Figure 36. On this day there were apparently two periods of heaviest attack; the first, during early morning; and the second, in late afternoon. When the maximum daily temperature was considerably lower and the nights were cool (below 70° F.), this type of trend was never observed but, on the contrary, one such as is shown in Figure 37. In this instance, the fly population increased gradually throughout the day. This type of trend was found to be especially prevalent during September. Although these trends may be considered typical, on certain days the fly trend assumed a form not easily explicable (Figure 38). The

FIG. 56. TYPICAL FLY POPULATION TREND DURING HOT WEATHER; FOURTH DAY OF PRELIMINARY COUNTS, SERIES II, 1934 (F=FLY POPULATION; T=TEMPERATURE)

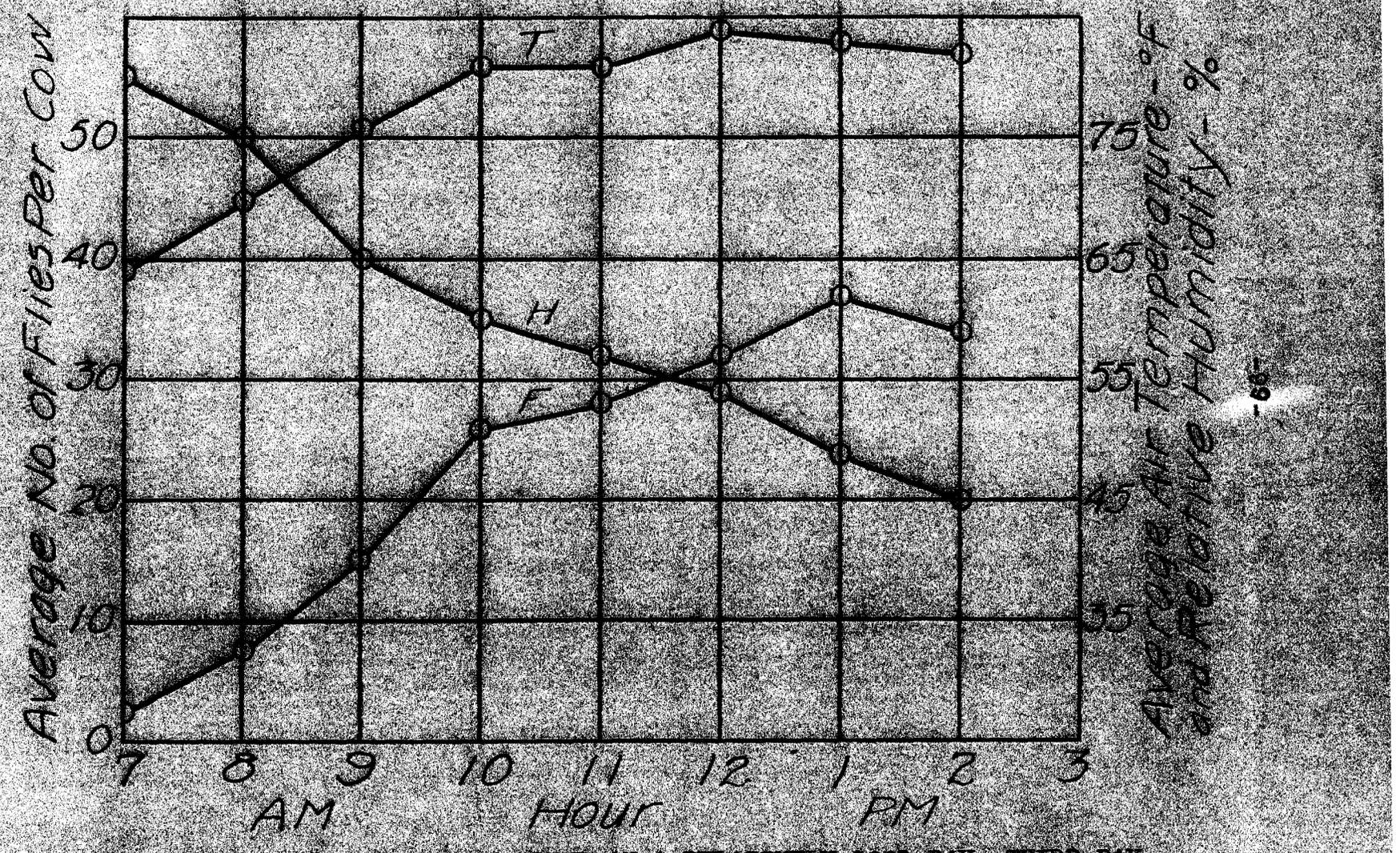

FIG. 37.- TYPICAL FLY POPULATION TREND ON A RELATIVELY COOL DAY; THIRD DAY OF PRELIMINARY COUNTS, SERIES IV, 1935 (F-FLY POPULATION; H. HUMIDITY; T-TEMPERATURE)

FIG. 38. AN UNUSUAL FLY POPULATION TREND; THIRD DAY OF PRELIMINARY PERIOD COUNTS, SERIES II, 1934 (F=FLY POPULATION; T=TEMPERATURE)

optimum range of temperature for fly attack varied from 70° to 90° F. Temperatures outside these limits were usually accompanied by a reduced or insignificant fly population. Humidity apparently affected the fly trend only when excessively high. The effect of slight precipitation is emphasized in Figure 39. Between eleven and one o'clock, a light rainfall occurred, indicated by a sharp rise in the humidity curve. Simultaneously the fly trend showed a corresponding increase. Apparently a number of flies, not on the cows at that time, took temporary refuge upon them during this interval. Opportunity was not provided to make observations of conditions during periods of extremely low humidity.

Physiological Effects

The physiological effects of fly sprays upon cattle have been studied by Melvin (1932), Wilson, et al. (1933) and Freeborn, et al. (1934). During this investigation, no detailed observations were made in this connection. However, precautions were taken to detect any possible skin injury or symptoms of distress attributable to the spray materials. By comparison with other cows in the herd, none of the sprayed animals displayed any apparent ill effects from any of the formulae. Pine oil No. 303, at 25% concentration in No. 40 base oil failed to produce any harmful results.

FIG. 39. A FLY POPULATION TREND AFFECTED BY A SUDDEN CHANGE IN HUMIDITY; SECOND DAY OF PRELIMINARY COUNTS, SERIES V, 1935 (F=FLY POPULATION; H=HUMIDITY; T=TEMPERATURE)

Discussion

During Series I and II, 1934, "No Spray" was compared with base oil No. 40 (alone), using unsprayed cows grouped according to their fly susceptibility averages. The data thus obtained are presented on a percentage efficiency basis in Table 37.

TABLE 37. EFFICIENCY OF BASE OIL NO. 40 (ALONE) AS A FLY REPELLENT.

Series	Per Cent Efficiency									
	Hour									Average
	7	8	9	10	11	12	1	2	3	
I	75	66	56	38	36	42	31	20	-	44
II	78	60	35	47	36	29	47	31	42	44

This base oil showed a range in efficiency from 20 to 78 per cent, with an average of 44 per cent in each series. These results indicate that if a given spray formula is to be compared with "No Spray" (as has been done in the past) the added repellent ingredient or ingredients should be given credit only for efficiency in excess of that produced by the base oil (alone). Consideration should also be given to the fact that base oil varies in efficiency with different cows. However, the large annual consumption of cattle fly sprays relegates the problem

of "Spray vs. No Spray" to a comparatively elementary position. The development of new cattle fly spray formulae, which are relatively more efficient and economical seems to be, at present, the most promising field for further research.

SUMMARY AND CONCLUSIONS

Toxicity Tests

Over nine hundred toxicity tests on house flies, Musca domestica L. were conducted in a modified "Peet-Grady" testing chamber. Only highly refined petroleum base oils were employed. The spray formulae included steam-distilled pine oils, alone, and in combination with pyrethrum, derris, rotenone and an aliphatic thiocyanate.

Pine oil is not sufficiently toxic to warrant its use as the sole added ingredient of a petroleum oil-base cattle fly spray. It exhibits greater toxicity when incorporated with a base oil of low viscosity (30-35 secs.) than with a base oil of high viscosity (85 secs.).

High grade pine oils are, in general, more toxic than those of low grade.

The toxicity of pine oil to house flies continues to manifest itself over a period of 48 hours after exposure.

Pine oil increases (activates) the toxicity of pyrethrum extract, in relation to the amount added. The toxicity of a 1 pound per gallon pyrethrum spray may be maintained by substituting 10, 15, or 25 per cent pine oil for 1/4, 1/2, or 3/4 pound pyrethrum, respectively.

Pyrethrum-pine oil combinations, in a petroleum oil-base, display the same rate of kill as a "pyrethrum alone" spray, there being no significant mortality after 24 hours.

Pyrethrum-pine oil combinations possess a higher "knock-down" value than a "pyrethrum alone" spray of comparable mortality.

Pine oil retards the rate of precipitation, change of color and loss in toxicity of pyrethrum sprays when exposed to sunlight.

High grade pine oils are more effective than those of low grade, for combination with pyrethrum, in respect to both toxicity and the prevention of deterioration.

Both the "knockdown" and mortality produced by derris extract are increased (activated) by pine oil. The rate of activation is greater than with pyrethrum.

Derris-pine oil combinations exhibit the same rate of kill as derris alone, significant mortality occurring after 24 hours.

High grade pine oils are more effective than those of low grade when combined with derris extract. The differences are not as distinct, however, as with pyrethrum.

The effect of pine oil upon the toxicity of rotenone and of derris extract is similar.

Pine oil in concentrations as high as 25 per cent produces no effect upon the toxicity of an aliphatic thiocyanate.

From the standpoints of efficiency and economy, none of the pine oils tested are superior to No. 303 in toxicity, or as an activator of pyrethrum and derris extracts.

Repellence Tests

Ten series of field repellence tests were conducted with cows, according to a "close observation" method. A highly refined petroleum base oil (No. 40) of 40-45 seconds viscosity (Saybolt, 100° F.) was employed in each series. The spray formulae consisted of a high grade, steam-distilled pine oil (No. 303), pyrethrum, derris, an aliphatic thiocyanate, and combinations of these materials with the pine oil (No. 303).

The repellent efficiency of a highly refined petroleum base oil (No. 40) apparently varies from 20 to 78 per cent over an 8 to 9 hour period. An average of 44 per cent was recorded in each of two series of tests.

Pine oil is not sufficiently repellent to warrant its use as the sole added ingredient of a petroleum oil-base cattle fly spray.

Pine oil increases the repellence of pyrethrum extract in relation to the amount added. The repellence of a one pound per gallon pyrethrum spray may be maintained by substituting 10 or 15 per cent pine oil for 1/4 or 1/2 pound pyrethrum, respectively.

Pine oil increases the repellence of derris extract in relation to the amount added, but not at as great a rate as that of pyrethrum extract.

The repellence of an aliphatic thiocyanate is unaffected by the addition of pine oil.

Cows sprayed with 25 per cent pine oil in a highly refined petroleum base oil (No. 40) suffered no skin injury or other ill effects from such treatment.

Pine oil may be safely and economically employed in practical cattle fly spray formulae.

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